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# Development of a Zero-Headspace Aerobic Suspended Growth Bioreactor

*by Mark E. Zappi, Robert T. Morgan, M. Todd Miller, WES*

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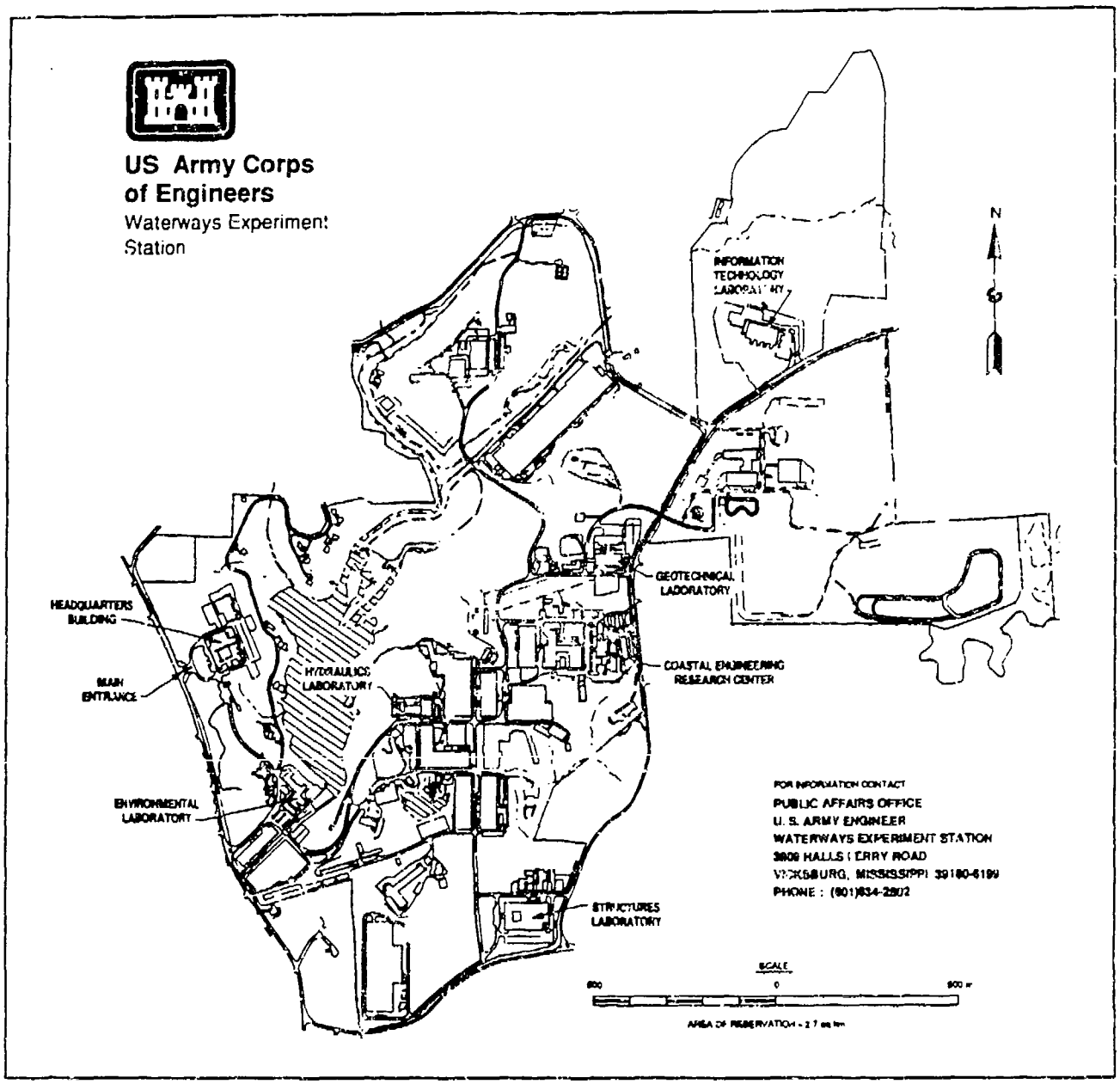


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# Preface

The work reported herein was conducted by the U.S. Army Engineer Waterways Experiment Station (WES) under funding from the In-House Laboratory Independent Research Program.

This report was prepared by Messrs. Mark E. Zappi, M. Todd Miller, and CAPT Robert T. Morgan, Environmental Restoration Branch (ERB), Environmental Engineering Division (EED), Environmental Laboratory (EL), WES, and Dr. Mohammad M. Qasim, Department of Chemistry, Shaw University, Raleigh, NC, while on sabbatical at WES under an Interagency Personnel Act.

This work was conducted under the direct supervision of Mr. Norman R. Francingues, Chief, ERB, and under the general supervision of Dr. Raymond L. Montgomery, Chief, EED, and Dr. John Harrison, Director, EL.

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# Conversion Factors, Non-SI to SI Units of Measurement

Non-SI units of measurement used in this report can be converted to SI units as follows:

| Multiply | By | To Obtain |
|---|---|---|
| acres | 4,046.873 | square meters |
| cubic yards | 0.7645549 | cubic meters |
| Fahrenheit degrees | 5/9 | Celsius degrees[1] |
| feet | 0.3048 | meters |
| gallons (U.S. liquid) | 3.785412 | liters |
| gauss | 0.0001 | tesla |
| inches | 2.54 | centimeters |
| miles (U.S. statute) | 1.609347 | kilometers |
| pounds (mass) | 0.4535924 | kilograms |
| tons (metric) | 1,000 | kilograms |
| tons (2,000 pounds, mass) | 907.1847 | kilograms |
| watts per square inch | 1,550.003 | watts per square meter |

[1] To obtain Celsius (C) temperature readings from Fahrenheit (F) readings, use the following formula: $C = (5/9)(F - 32)$.

# 1 Introduction

## Background

The U.S. Department of Defense (DoD) has numerous sites that are contaminated with organic compounds. These chemicals threaten the overall integrity of one of the country's most valuable resources—groundwater. Unfortunately, extensive groundwater contamination has already occurred at many of these sites, requiring that some form of groundwater remediation be initiated. Recent estimates indicate that the DoD has responsibility for cleanup of an estimated 12,000 sites. Cost estimates for the cleanup of 12,000 sites using traditional treatment technologies are astronomical. Recent regulatory trends have further compounded the economic and technical dilemma facing the DoD with respect to the vast cleanup effort it must undertake.

Traditional technologies for treatment of organics-contaminated groundwater, such as activated carbon and air stripping, can be cost prohibitive and do not result in the onsite destruction of the contaminants. Activated carbon and air stripping simply result in the transfer of contaminants from the liquid phase onto the solid phase. Current costs associated with the implementation of these technologies range from $0.75 to $10.00 per thousand gallons treated.

Innovative treatment technologies based on physical, chemical, and biological processes offer remediation alternatives that are technically, economically, and politically more attractive. Among these innovative technologies, biological treatment systems (biotreatment) are considered to be one of most economically and technically promising. Cost estimates for groundwater biotreatment processes tend to fall within the lower bounds of the same cost range presented for activated carbon and air stripping, yet biological processes provide onsite destruction of the contaminants.

## Biotreatment Processes

Biological treatment (biotreatment) of organic contaminants has recently enjoyed much popularity as a potential treatment technology for remediation of contaminated sites. Although biotreatment is a promising technology, it is not a panacea. Knowledge of the current positive and negative aspects of all

configurations of biotreatment must be identified before the technology can be properly implemented. Biotreatment technologies can be divided into two main implementation categories: aboveground and in situ. Use of aboveground systems requires excavation of the contaminated soils or extraction of contaminated groundwater followed by treatment in aboveground reactors. Examples of such systems include activated sludge, sequential batch reactors, and bioslurry reactors. Treatment of contaminated aquifers does not always require excavation of soil. Pump-and-treat systems involve removal of the contaminants from the soils via desorption into the groundwater followed by treatment of the groundwater in an aboveground bioreactor. The treated groundwater is then injected back into the aquifer for recirculation through the contaminated portion of the aquifer.

## Activated Sludge Fundamentals

Activated sludge is a suspended growth biotreatment technology that has been used extensively in the United States for treatment of municipal wastewaters. The design and operation of aerobic, suspended growth bioreactors for municipal wastewater treatment is well documented (Metcalf and Eddy 1991; Sundstrom and Klei 1979). The technology has also seen some usage as a means of treating contaminated industrial wastewaters and groundwaters.

Treatment of industrial wastewaters containing similar compounds found in many contaminated groundwaters is well documented (Nayar and Sylvester 1979; Wong and Goldsmith 1988; Kincannon et al. 1989), indicating a high potential for use of activated sludge as a means of treating contaminated groundwater. Biological treatment of contaminated groundwaters containing volatile organic compounds (VOCs) using activated sludge systems has been successfully accomplished by a variety of researchers (Weber and Jones 1986; Zappi et al. 1990; Zappi, Teeter, and Francingues 1990; Folsom and Chapman 1991; ABB Environmental Inc. 1991; Speitel and Leonard 1992). Unfortunately, activated sludge systems utilize sparging of air or pure oxygen as an oxygen source for the microorganisms, which can result in significant volatilization (via stripping) of VOCs into the process gas stream exiting the reactor. If the levels of VOCs in the process gases exceed contaminant release criteria governing the site, then costly treatment of the process gases are usually required.

Activated sludge (AS) is a biological process that utilizes acclimated bacteria for the aerobic degradation of contaminants in wastewater. Figure 1 is an illustration of a typical AS treatment system. The term "acclimated" means that the bacteria consortium utilize the organic contaminants in the influent as their food source. The populations of bacteria in the aeration tank are so great that the air-activated organic biological solids made up primarily of dense colonies of bacteria are referred to as AS. The AS/wastewater slurry in the aeration tank is commonly known as the mixed liquor (ML). The ML is kept in suspension in the aeration tank by using either mechanical mixers or

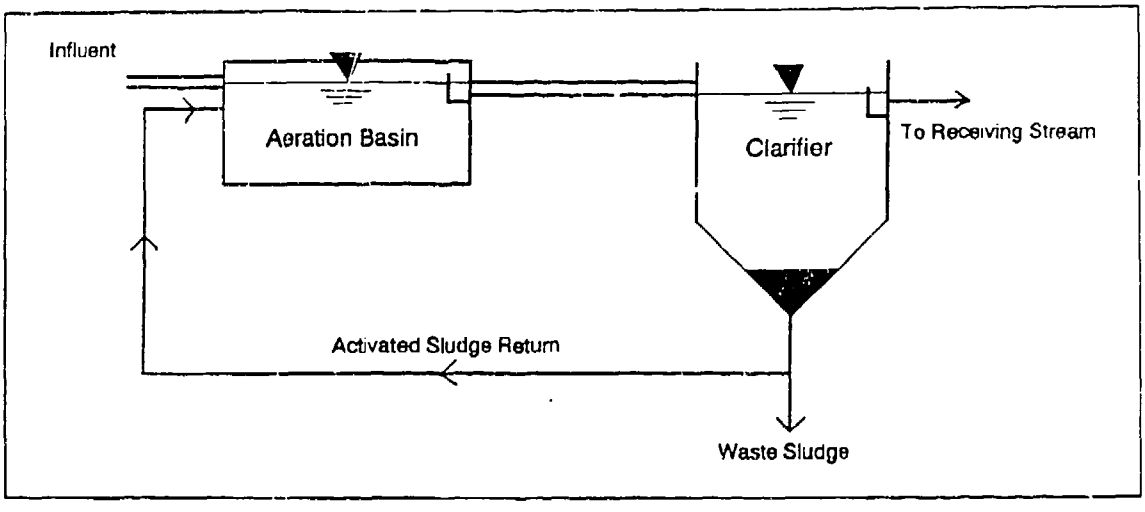



Figure 1. Schematic of a typical activated sludge system

diffused air. Since biological solids are volatile, bacterial populations in the ML are often measured using ML suspended solids concentrations (MLSS), usually presented as mg SS/ℓ ML, or ML volatile suspended solids concentration, usually presented as mg VSS/ℓ ML (MLVSS). Indicative of how highly populated AS systems are with active biomass is the use of solids analysis as an accurate measurement of overall microbial populations. AS systems are one of the most densely populated biological treatment systems utilized in the pollution remediation industry. These systems are orders of magnitudes (i.e., $10^{10}$ - $10^{12}$) higher in populations of microorganisms than other biotreatment technologies such as bioslurry ($10^5$ - $10^9$) or in situ biotreatment ($10^4$ - $10^8$).

## Hydraulic retention time (HRT)

The contaminated water fed into the bioreactor, referred to as influent, is added to the aeration tank at a rate that is carefully controlled to achieve a specific hydraulic retention time (HRT). HRT has units of time and theoretically describes the amount of time a particle of water is retained in the aeration tank. HRT is calculated as follows:

$$HRT = V_r/Q_{in}$$

where

HRT = hydraulic residence time, hr

$V_r$ = reactor volume, gal

$Q_{in}$ = influent flow rate, gal/hr

HRT is a process control parameter that essentially determines the amount of contact time between the contaminants and the microbial consortia. The appropriate HRT for a given bioreactor system is highly dependent on contaminant biodegradability and concentration, target treatment goals, and settling characteristics of the ML. Municipal wastewater plants typically treat relatively easy-to-degrade carbon sources; therefore relatively short HRTs are used (4 to 8 hr). Since contaminated groundwaters typically contain carbon sources that are much more difficult to degrade than municipal water sources, HRTs on the order of 12 to 48 hr are not uncommon. During the acclimation phase, close observation of gross pollutant removal, MLSS, MLVSS, and sludge volume index (SVI) can be used to adjust HRT accordingly. The shorter the HRT, the smaller the bioreactor requirements. Reduced bioreactor tankage relates to reduced capital and/or operations costs.

HRT is also a good means of controlling the food-to-microorganism (F/M) ratio of the bioreactor system. This important ratio essentially is a measure of the organic loading exerted by a given mass of microorganisms within the reactor. Appropriate F/M ratios range from 0.1 to 0.6 based on wastewater biochemical oxygen demand (BOD) and are calculated by (Sundstrum and Klei 1979)

$$F/M = \frac{[BOD]_i}{SRT \times X}$$

where

$[BOD]_i$ = initial BOD concentration, mg/ℓ

SRT = sludge retention time, days

$X$ = MLVSS, mg/ℓ

In terms of groundwater treatment, F/M is of interest for maintaining adequate ML solids concentrations. If HRT is too high and F/M too low, then the bioreactor will not be able to support appreciable biomass populations because of low organic loading. Maintaining MLVSS concentrations in excess of 1,000 mg/ℓ is attempted during bench-scale studies for groundwater treatment; however, appropriate contaminant removal efficiencies must be maintained (i.e., complex organics may take quite some time to degrade) while keeping HRT as low as possible. This phase of treatability testing becomes less of a science and more of an art.

## Sludge retention time (SRT)

As fresh influent enters the aeration tank, treated water or effluent flows out of the aeration tank into some form of solids separation. The vast majority of AS systems use clarifiers, although the use of filtration devices such as membrane filters have recently received much attention as potential alternatives to clarifiers. A clarifier is a density-based sedimentation tank used to separate the biosolids from the effluent. To keep a constant population of bacteria in the aeration tank, a portion of the thickened sludge (settled sludge) is returned to the aeration tank. Since the bacteria are constantly reproducing, some of the thickened sludge must be wasted from the bottom of the clarifier or directly from the aeration tank to maintain a constant bacterial population in the aeration tank. The amount of bacteria wasted periodically is based on the solids retention time of the biological solids. The SRT or sludge age is theoretically equal to the amount of time a particle of solid matter (bacteria) remains in the aeration tank. SRT is calculated by

$$\text{SRT} = \frac{(\text{VOL}) \times X}{(X_r \times Q_{ws}) + (X_e \times Q_e)}$$

where

SRT = sludge retention time, days

$X$ = ML solids concentration (MLSS or MLVSS), mg/ℓ

$X_r$ = solids concentration of waste sludge, mg/ℓ

$Q_{ws}$ = daily waste sludge flow, gal/day

$X_e$ = effluent solids concentration, mg/ℓ

$Q_e$ = effluent flow rate, gal/day

This process control parameter is often used to fine-tune bioreactor performance. SRT impacts both sludge settling characteristics, often defined by SVI, and microbial populations (MLSS or MLVSS). Typical SRTs range from 4 to >20 days (extended aeration).

SRT may also impact the degradational ability of the biomass. If possible, evaluation of various SRTs using multiple bioreactors can generate data that can be used to determine several kinetic constants. Evaluation of various SRTs will also allow for evaluation of the impact of SRT on contaminant removal and SVI. However, determination of these constants is not absolutely necessary, but can be very useful in later process operations.

SRT can also be used for controlling F/M ratio. Extended aeration is an operational regime where SRTs in excess of 20 days are used to dispose of

sludge within the bioreactor itself. The concept is to allow the biomass to operate well into the endogenous decay phase. During endogenous decay, the biomass feeds upon itself, thereby potentially eliminating sludge disposal requirements. Small AS plants (less than 0.1 million gallons per day (Mgd)) treating municipal wastewater are often operated within the extended aeration range. Many groundwater remediation systems may also be operated within extended aeration range.

## Treatment of Influents containing complex organics

The suitability of AS for the degradation of a variety of complex xenobiotic compounds has been demonstrated by many researchers (Kim, Humenick, and Armstrong 1981; Venkataramani and Ahlert 1984; Kelly 1987). Most of the research activities reviewed generally used the same technical approach to evaluate biological treatment that was used in this study. That approach is that a microbial consortium containing an extremely diverse variety of microbial types, such as ML from an AS system, are slowly exposed to the contaminants in the test influent. The chance of success is high because microbial populations capable of completely mineralizing the contaminants are usually present in AS.

Sanford and Smallbeck (1987) used a mixed consortium of bacteria and yeast to degrade a synthetic wastewater comprised of 100 ppm acetone, 50 ppm 2-butanone, and 125 ppm methyl isobutyl ketone in bench-scale chemostats. They concluded that treatment of ketones was successful within 48 hr of batch treatment utilizing a stable consortium of microorganisms and yeast.

Kim and Maier (1986) evaluated the acclimation and biodegradation potential of chlorinated organic compounds in the presence of cometabolites. They were able to acclimate a consortium of bacteria from a municipal AS plant capable of degrading 2,4-D (2,4 dichlorophenoxyacetic acid) and 3,5-DCB (3,5 dichlorobenzoate) under aerobic conditions. Combined contaminant concentrations as high as 100 mg/ℓ were successfully degraded. They concluded that seed bacterial consortia should contain as diverse a population of microorganisms as possible to increase the probability of plasmid exchange, and that the acclimation phase should begin with an influent containing very low concentrations of the target compounds to avoid inhibitory effects.

Bieszkiewicz and Pieniadz-Urbaniak (1984) evaluated the effect of benzene and xylene at concentrations as high as 75 and 150 mg/ℓ, respectively, on the work of an AS system. They concluded that increased concentrations of the target compounds generally decreased the chemical oxygen demand (COD) removals, increased SVI, increased the number of bacteria, and finally, altered the morphology of the bacterial flocs.

Rozich and Gaudy (1985) evaluated the response of an AS system to quantitative loadings of phenol. Phenol concentrations of 500 mg/ℓ were evaluated

as a base influent concentration. Initially, shock loadings of 1,000 mg/ℓ of phenol were imposed on the system without significant disturbances in treatment occurring. The AS system was then shocked with 2,000 mg/ℓ of phenol, which resulted in a collapse of the stability of the AS system. They concluded that design engineers should design AS systems that will be treating possible inhibitory and/or toxic compounds with high SRTs, especially systems that could be subjected to periodic shock loadings of contaminants.

Zappi et al. (1990) and Zappi, Teeter, and Francingues (1990) evaluated the feasibility of using AS and AS with powdered activated carbon addition for treatment of a highly contaminant groundwater from a Superfund Site. Their results indicated that AS was an economical and technically attractive option for treatment of the groundwater. The results of that study were used in the design of a full-scale treatment system that is currently meeting all design objectives. As predicted by the bench-study results generated by Zappi et al. (1990), the full-scale unit currently does have to treat the off-gases from the American Petroleum Institute (API) separator and the aeration vessels of the bioreactor system.

## Electron Acceptors in Biological Treatment

All biological treatment processes are essentially engineered, ecological systems that are managed via process controls to provide those environmental conditions that result in targeted beneficial reactions, performed by microorganisms, required for completion of design objectives. In terms of AS, the microorganisms of prime importance are bacteria. Bacteria are single-celled organisms within which literally thousands of independent enzyme-catalyzed biochemical reactions take place. Most organic contaminants undergoing biological treatment today are metabolized via enzymatic-based oxidation reactions that use molecular oxygen as the electron acceptor. During the metabolic degradation of organic contaminants (also called substrates) within bioreactors, two phases of microbial reactions occur. The first phase involves the oxidation of the substrate to carbon dioxide or some lesser carbon source and the transfer of the electrons, $e^-$ (i.e., hydrogen atoms), to oxidized forms of nucleotides such as nicotinamide adenine dinucleotide (NAD). The second phase of respiration is the transfer of hydrogen atoms through a variety of transfer sequences that ultimately results in the regeneration of NAD accompanied by conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). ATP is the energy carrier in all living cells. The hydrogen atoms are ultimately reacted with oxygen to form water. An overall reaction that describes aerobic cell respiration based on NAD liberation is presented below (Bailey and Ollis 1986):

$$NADH + H^+ + 0.5O_2 + 3ADP + 3P_i \rightarrow NAD^+ + H_2O + 3ATP$$

The oxygen in this reaction serves as the electron acceptor (i.e., receives hydrogen); therefore, during aerobic degradation of contaminants, oxygen is often referred to as the terminal electron acceptor. However, other less energy- efficient electron acceptors can be used by microorganisms. From an electron acceptor usage standpoint, bacteria may be divided into three categories: obligate anaerobes (survive only in the absence of oxygen), obligate aerobes (must have oxygen to survive), or facultative organisms (can survive with and without oxygen).

Nitrates, sulfates, and carbon dioxide are known acceptors of electrons in microbial processes. In the absence of oxygen, consortia capable of using alternative electron acceptors dominate the physiological profile of the consortia. The oxidation-reduction potential (REDOX) determines the dominant electron acceptor which has the order $NO_3 > SO_4 > CO_2$. In nature, the oxides of iron and manganese also act as electron acceptors, but their total contribution to net metabolic flux is limited (Lovel 1991).

Nitrate is an efficient electron acceptor. Wilson et al. (1986) successfully used nitrate as an electron acceptor for biodegradation of toluene, ethylbenzene, and xylenes. Since nitrate again has high solubility and a rather low interaction with soil particles, necessary quantities can be easily introduced in the aquifer, provided nitrate-utilizing bacteria have had a chance to develop in the system. This often requires anoxic conditions. Nitrate was considered as a possible electron acceptor for evaluation during this study. However, nitrate is considered by regulatory entities as a contaminant; as such, it is regulated. Because of concerns over difficulties in ensuring that inappropriate nitrate levels are not present in the bioreactor effluents, nitrate was eliminated from further consideration for potential inclusion into the study as a candidate electron acceptor. Sulfate and carbon dioxide were not considered practical sources of electron acceptors because of the low REDOX conditions that must be effectively maintained within a well-mixed bioreactor. As such, focusing on the exclusive development of an aerobic bioreactor system was decided. Also, aerobic bioreactor systems usually have much more rapid biokinetics than do anaerobic systems.

Oxygen is typically supplied to activated systems using gas sparging systems for injection of air (aeration), and in some cases, oxygen. Design calculations for most AS systems result in the sizing of aeration systems that incorporate most of their energy toward cell suspension and not cell respiration (i.e., bacterial oxygen demand). In other words, most of the air sparged into an AS bioreactor is used for keeping the aeration chamber contents well mixed and not for oxygen consumption by the bacterial consortia in the ML.

Flux requirements of molecular oxygen required for maintenance of the bacterial consortia in the ML may be determined through a simple respirometric technique referred to as oxygen uptake rate (OUR). OUR is a simple test that involves determination of the oxygen utilization rate exerted by a small microcosm over a given time increment (Note: these authors use 1 hr). The units of OUR can be presented as mg $O_2$/ℓ/hour. When OUR is presented

using these units, it represents a term that is bioreactor specific. OUR can also be presented based on MLSS or MLVSS using the units of mg $O_2/\ell/[MLSS \times hour]$, thereby, making OUR a bacterial population dependent term that gives the term some degree of freedom from reactor specificity. Design engineers must ensure that the bioreactor does not become oxygen limited. An approximation for aerobic biotreatment processes is that bioreactor dissolved oxygen (DO) levels in excess of 2.0 mg/ℓ should be maintained at all times. To accomplish this the oxygen delivery system, usually an aeration system, is designed to meet the expected maximum OUR required by the AS system. As discussed earlier, in the case of AS, the design of an aeration system is usually governed by mixing energy requirements and not OUR. If a mechanical mixer is employed to keep the ML mixed, then an oxygen delivery system can be sized based solely on the expected maximum bioreactor OUR.

A variety of sources of molecular oxygen are available to the design engineer. Popular sources that have been used in AS are diffused air (by far, the most popular) or oxygen (Metcalf and Eddy 1991). Solid and liquid forms of hydrogen peroxide have been used as oxygen sources during biotreatment; however, these applications have basically been limited to in situ biotreatment systems for remediation of contaminated aquifers (Bajpai and Zappi 1994).

## Hydrogen Peroxide - Bacteria Interactions

Hydrogen peroxide ($H_2O_2$), discovered in 1818 by Louis-Jacques Thenard, is a clear, colorless, slightly viscous liquid that is completely miscible with water at any proportion. It can be produced using a variety of manufacturing processes including thermal, electrolytic, sonic, or irradiation-based processes (Schumb, Satterfield, and Wentworth 1955). The densities of 30-, 50-, and 100-percent (pure) hydrogen peroxide solutions are 1.1081, 1.1914, and 1.4425 g/ml, respectively, at 25 °C.

Hydrogen peroxide is often used in the chemical manufacturing industry as an oxidizer and reducer depending on process requirements. It has been used commercially for bleaching, chemical synthesis, weapons propulsion, and disinfection. It can be easily transported to reactor siting locations because of its high degree of stability. Hydrogen peroxide has recently received much attention as a hydroxyl radical production source during ultraviolet radiation for groundwater treatment (Sundstrom et al. 1986; Zappi, Teeter, and Francingues 1990; Zappi et al. 1990) and has also been used as a disinfection agent (Block 1991). It provides a molecular oxygen source that stoichiometrically contains approximately 94-percent oxygen by weight. However, since the most common commercial forms of hydrogen peroxide are 30 and 50 percent (wt/wt), then these formulations essentially represent approximately 28- and 47-percent available oxygen, respectively, if these formulations are supplied without further dilution.

Microbial enzymes are the biochemical catalysts that initiate all key biochemical reactions within the cell. The oxidation of a substrate presented

earlier is catalyzed by a group of enzymes referred to as oxidases. Biological enzymes that catalytically decompose hydrogen peroxide and thereby eliminate it from the biochemical system are called catalases (Schumb, Satterfield, and Wentworth 1955). This enzyme is found in all plants and animals. Of all living organisms, only a few microorganisms do not contain catalase-production capability. Catalases are used by organisms to prevent toxic levels of hydrogen peroxide from accumulating within cells. Hydrogen peroxide is formed in living systems through reduction of oxygen to hydrogen peroxide via aerobic dehydrogenases (Schumb, Satterfield, and Wentworth 1955). The decompositional capability of catalase is great from a hydrogen peroxide-to-catalase stoichiometric ratio prospective. Catalase is considered by far the most effective at hydrogen peroxide destruction of all chemical processes known. The mechanism of hydrogen peroxide decomposition by catalase is termed "catalatic" to distinguish it from peroxidatic activity (Schumb, Satterfield, and Wentworth 1955). Peroxidase is another enzyme with hydrogen peroxide degradational capability, however, at a much lower extent and occurrence.

Both catalase and peroxidase are considered hemoproteins. These enzymes are likely to be the predominant hydrogen peroxide destruction mechanisms within bioreactor systems. They are made up of a protein bearing an active or prosthetic group typified as an iron protoporphyrin (Schumb, Satterfield, and Wentworth 1955). Catalase and peroxidase are structurally similar to other biological enzymes such as hemoglobin, which is a component of red blood cells that carries oxygen, and myoglobin, a component of muscles that stores oxygen. Although these enzymes are similar, they are significantly different in carrying out different biochemical functions.

Hydrogen peroxide is not found within the cell walls of aerobic organisms because of the presence of catalase within these cells (Schumb, Satterfield, and Wentworth 1955). Hydrogen peroxide has been detected on the walls of inactivated obligate anaerobes that were exposed to oxygen, indicating the formation of hydrogen peroxide after exposure. Anaerobes are usually devoid of catalase-production capability and are especially susceptible to the toxic effects of hydrogen peroxide at very low concentrations. Since anaerobes tend to produce highly odorous by-products during metabolism, this low tolerance is one of the reasons why hydrogen peroxide is added to sewerage plants that are emitting excessively high amounts of odor compounds. Hydrogen peroxide is also added to sewage plants to quickly increase residual oxygen levels.

Hydrogen peroxide has been used in numerous cases as an oxygen source during in situ biotreatment of contaminated soils (Zappi et al. 1993). DO is released from hydrogen peroxide because of its hydrolysis, which is mediated by metals found in the soil matrix or through enzymatic-based degradation via catalases and peroxidases produced by microorganisms (Bajpai and Zappi 1994). These enzymes are expected to be abundantly present in all systems containing aerobic microbial activity. As a result, hydrogen peroxide introduced into aqueous bioreactor systems will probably degrade rapidly into water and oxygen. Once oxygen is produced, the oxygen is then utilized by aerobic

microorganisms for respiration. Spain et al. (1989) evaluated hydrogen peroxide as an oxygen source for an in situ biotreatment project in northern Florida. They concluded that enzymatic degradation via catalase activity was too significant to maintain appropriate oxygen levels. They determined that the rate of hydrogen peroxide was first order with a half-life of 4 hr when using a 500-mg/ℓ hydrogen peroxide dose added to a 3:1 water:soil (wt/wt) slurry under agitation. They suspected that iron-catalyzed degradation of hydrogen peroxide was occurring (commonly referred to as Fenton's Reaction). After adding a commercial phosphate-based hydrogen peroxide stabilizer, they observed a three-fold increase in hydrogen peroxide half-life. However, when hydrogen peroxide was injected into an infiltration gallery that was pretreated to prevent iron-based reactions, poor hydrogen peroxide transport was observed, indicating a biologically based decomposition. Subsequent studies were performed comparing the fate of hydrogen peroxide in autoclaved, mercuric chloride, and untreated soil slurries. Their results clearly indicated that the primary degradational hydrogen peroxide mechanism was biologically based and was presumed to be catalase based.

Microbial-mediated degradation of hydrogen peroxide is considered a major concern for this study because if the rate of microbial degradation of hydrogen peroxide is too rapid, then a consistently sufficient residual oxygen concentration within the bioreactor may not be maintained. If excessively high amounts of hydrogen peroxide must be added to achieve acceptable oxygen levels then it may be cost prohibitive toward application as an alternative to gas sparging.

Another aspect of delivery of oxygen in the form of hydrogen peroxide into a bioreactor that needs to be considered is that hydrogen peroxide at increased levels is toxic to many microorganisms. Hydrogen peroxide has found significant usage as a disinfection agent primarily because it degrades to water and oxygen after exerting its toxic effect on microorganisms (Block 1991). Hydrogen peroxide is also found in human saliva as a natural disinfecting agent. It appears to have more disinfecting activity against bacteria and viruses than fungi and appears to have greater activity against gram-negative organisms than gram-positive (Block 1991). Its disinfecting activity is pH dependent with increased activity occurring at acidic conditions. Curran, Evans, and Leviton (1940) found the greatest disinfecting potential at a pH of 3 and the lowest at a pH of 9 for bacillus spores at a dosage of 1 percent and 50 °C. Toxic concentrations of hydrogen peroxide reported in published literature range from 100 to >1,200 ppm (Schumb, Satterfield, and Wentworth 1955; Block 1991). Schumb, Satterfield, and Wentworth (1955) present evidence that levels on the order of 0.0003 and 0.0027 percent (wt/wt) were inhibitory to growth and toxic, respectively, toward *staphylococcus aureus*. Although there is no direct evidence, this large diversity may be a reflection of catalytic activity of the various bacterial consortia in the different experiments. However, it is apparent that aerobic consortia do contain antihydrogen peroxide activity that can be overcome through overdosing the biological system with excessive amounts of hydrogen peroxide.

## Study Objectives

The primary objective of this study was to evaluate the feasibility of using a nongaseous oxygen source as an alternative to air or pure oxygen for maintenance of biological systems; AS was used for this study, treating an influent containing high levels of VOCs. Feasibility was gaged by the following requirements:

*a.* Minimum or no reduction of bioreactor effectiveness in terms of treatment efficiency.

*b.* Appreciable reductions in VOC off-gassing from the AS reactor.

*c.* Minimal increase in bioreactor operational and maintenance costs.

# 2 Experimental Methods

## Summary of Experimental Approach

The objectives of this study were met by performance of the following major study phases:

*a.* Acclimation Phase - This phase involved acclimation of a consortium of bacteria to the contaminants (benzene) in the groundwater using ML from an AS system as the initial bacterial seed source. This phase lasted from Test Day 1 through Day 181 (i.e., 6 months).

*b.* Hydrogen Peroxide Introduction Phase - This phase focused on converting one of the three bioreactors to using hydrogen peroxide as an alternative oxygen source as opposed to sparged aeration. This phase was considered the most crucial phase of study because if conversion to hydrogen peroxide was not possible, then the overall hypothesis of this study was not correct (i.e., using a liquid oxygen source to reduce or eliminate VOC off-gassing during aerobic biotreatment). This phase lasted from Test Day 182 through Day 329 (i.e., 5 months).

*c.* Comparison Phase - This phase involved using three replicate 3-ℓ AS bioreactors with equal organic loadings to compare three candidate sources of oxygen: sparged air, sparged pure oxygen, and injected liquid hydrogen peroxide. Bioreactor performance was compared based on contaminant removal, biomass stability, reduced VOC off-gassing, and cost analysis. This phase lasted from Test Day 329 through Day 523 (i.e., 6.5 months).

*d.* Hydrogen Peroxide Feed Optimization - This phase focused on determining an optimal hydrogen peroxide dose for reducing reactor off-gas VOC concentrations. This phase lasted from Test Day 524 through Day 546 (i.e., 0.75 months).

## Study Influents

Test influents used in this study were groundwater samples collected from Observation Well 01014 located at Rocky Mountain Arsenal (RMA), Commerce City, Colorado, and wastewater influent from the City of Vicksburg, Mississippi, Public Owned Treatment Works (VPOTW). The Acclimation Phase of study was the only period in this study that utilized the VPOTW influent. The other study phases utilized amended RMA groundwater as the sole influent source. The VPOTW wastewater was used to initiate bioreactor operations and maintain bioreactor activity during acclimation of microbial consortia within the ML with the contaminants in the groundwater.

The VPOTW influent was collected after primary treatment (treated using primary sedimentation) and before introduction into two trickling filters. Since primary treatment tends to remove approximately 50 percent of the BOD and reduces nutrient levels, the wastewater was amended with nutrients to formulate a system influent that contained appropriate levels of carbon substrate and nutrients (discussed later).

The major contaminant present in the groundwater sample was benzene ($C_6H_6$), which over the course of this study had an average concentration of 200 mg/ℓ. Figure 2 presents the influent BOD, COD, and benzene levels measured within study influents throughout the course of this study. This figure presents analytical data from all of the study phases. The groundwater samples were collected at RMA on several occasions throughout the study in 55-gal[1] drums and returned to the U.S. Army Engineer Waterways Experiment Station (WES) for storage in a walk-in cooler maintained at 4 °C until needed. Changes in influent strength were observed over time as the samples aged and volatile contaminant losses occurred in the drum headspace (which increased as sample was removed). Other contaminants were present at much lower levels than the benzene. The only other major contaminant present in the groundwater was dichloropentadiene (DCPD), which was detected at levels <10 mg/ℓ. DCPD is not volatile and should not have contributed to bioreactor contaminant off-gassing.

Table 1 lists the feed proportions of the VPOTW influent to groundwater composite over the acclimation period to shift the influent composition from VPOTW influent to groundwater. The table also lists a variety of influent-related information on chemical composition and flow rate that will be discussed later. Over an approximate 4-month period, the influent to the acclimation bioreactor was proportionally switched from VPOTW influent to the RMA groundwater. Within a 16-week period, the influent was switched from 100-percent VPOTW influent to 100-percent amended RMA groundwater. The rate at which the proportion of groundwater to VPOTW influent

[1] A table of factors for converting non-SI units of measurement to SI units is presented on page vii.

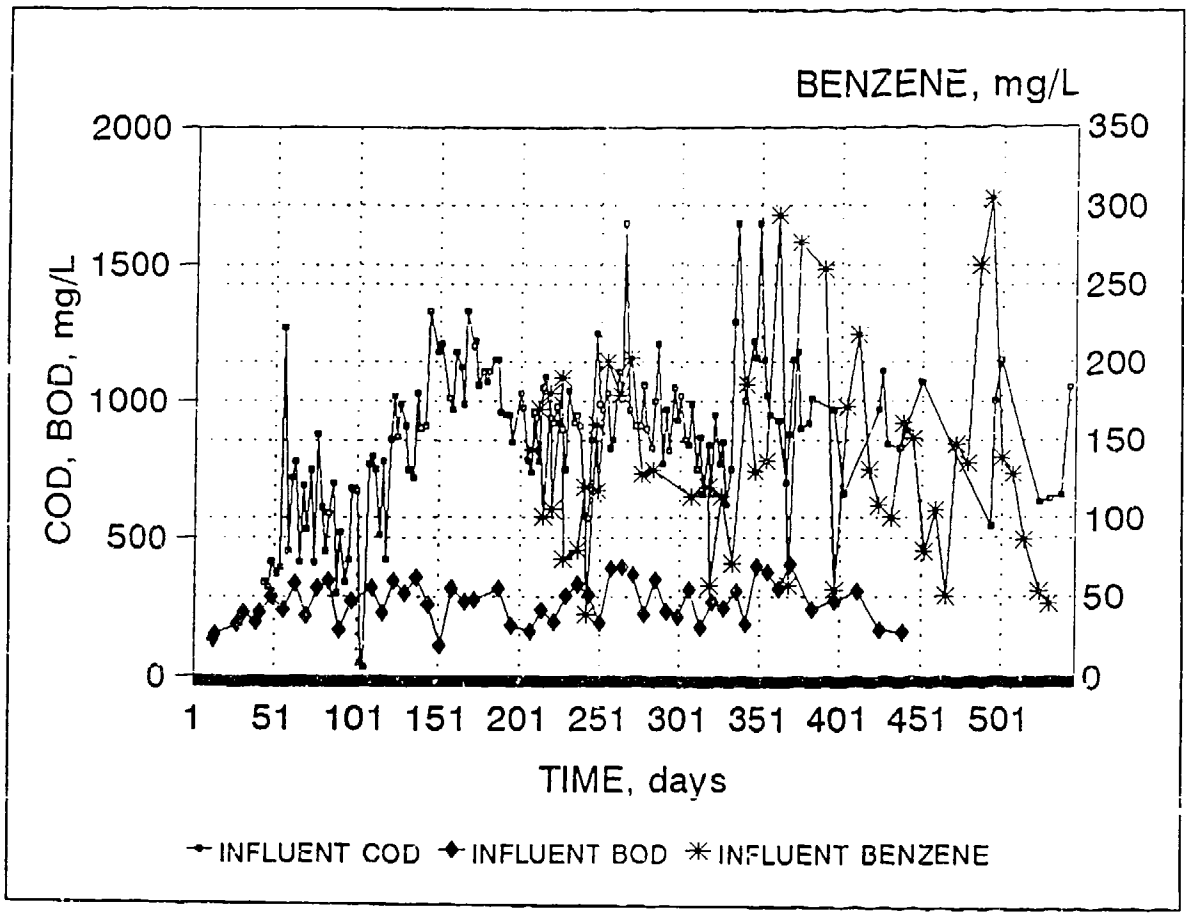



Figure 2. Influent chemical quality over the course of the study

was increased was determined before testing began under the assumption that the bacterial consortium would easily acclimate to the groundwater, with little or no lag phase, when maintained under a relatively constant system organic carbon loading through adjustments in amendment dosing amounts (Table 1). During the transitional period when groundwater was replacing the VPOTW influent, changes in gross contaminant concentrations (COD and BOD) were monitored at least weekly; MLVSS and MLSS levels were monitored at least three times a week. This was done to ensure that the contaminants in the groundwater were not adversely affecting biological activity. If adverse effects are observed, for example, significant changes in gross pollutant removal or dramatic decreases in the solids concentration, these effects could be reversed or minimized by decreasing the rate of groundwater addition.

## Bench-Scale Bioreactors

The bioreactors used in this study were the chemostat-type units illustrated in Figure 3. This bioreactor design is representative of AS systems and is commonly used for performing bench studies of this type (Zappi and Morgan 1993).

**Table 1**
**Influent Chemical Composition and Feed Rates Throughout Study (all study phases)**

| Test Day | GW % | Q mpm | HRT hr | [NaAC] g/ℓ | [AmSul] g/ℓ | [AmPh] g/ℓ | [Dex] g/ℓ | [Glu] g/ℓ | Comment on Action |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 6 | 5.6 | 0.3 | 0.24 | 0.02 | 0.03 | 0.04 | – |
| 26 | 7 | 6 | 5.6 | 0.3 | 0.24 | 0.02 | 0.03 | 0.04 | 1 |
| 42 | 27 | 6 | 5.6 | 0.3 | 0.24 | 0.02 | 0.03 | 0.04 | 1 |
| 47 | 27 | 6 | 5.6 | 0.4 | 0.24 | 0.02 | 0.07 | 0.07 | 2 |
| 59 | 40 | 6 | 5.6 | 0.67 | 0.24 | 02 | 0.2 | 0.2 | 1,2 |
| 82 | 53 | 6 | 5.6 | 0.67 | 0.24 | [illegible] | 0.2 | 0.2 | 1 |
| 87 | 67 | 6 | 5.6 | 0.67 | 0.24 | 0.02 | 0.2 | 0.2 | 1 |
| 91 | 67 | 3 | 11.0 | 0.67 | 0.24 | 0.02 | 0.2 | 0.2 | 3 |
| 96 | 67 | 2 | 17.0 | 0.67 | 0.08 | 0.02 | 0.2 | 0.2 | 4 |
| 108 | 87 | 2 | 17.0 | 0.67 | 0.08 | 0.02 | 0.2 | 0.2 | 1 |
| 110 | 87 | 2 | 17.0 | 0.67 | 0.48 | 0.04 | 0.2 | 0.2 | 5 |
| 122 | 100 | 2 | 17.0 | 0.67 | 0.08 | 0.02 | 0.2 | 0.2 | 4 |
| 136 | 100* | 2 | 17.0 | 0.103 | 0.27 | 0.012 | 0.03 | 0.03 | 6 |
| 229 | 100 | 2 | 17.0 | 0.185 | 0.27 | 0.012 | 0.06 | 0.06 | 2 |

Notes:
GW = groundwater.
Q = flow rate of influent into the bioreactors.
HRT = hydraulic retention time.
NaAC = sodium acetate.
AmSul = ammonium phosphate.
AmPh = ammonium phosphate.
Dex = dextrose.
Glu = glutamic acid.

Comments on Actions - The following numbers correlate to an explanation of why a particular system influent composition or dosing change occurred.
1 = Increasing the percentage of GW in the influent as part of the acclimation process.
2 = Increased the organic loading on bioreactors to stabilize MLSS and MLVSS.
3 = Decreased influent flow rate because of poor sludge settling. Lowering flow rate reduces the surface overflow rate of the in-line clarifier, which in turn reduces the potential for sludge washout.
4 = Higher levels of ammonia were found in effluents, therefore, input ammonia loading was reduced.
5 = Poor sludge settling and slightly decreasing MLSS and MLVSS prompted an increase in nutrient loading into the bioreactor to evaluate if the bioreactors may be slightly nutrient limited.
6 = Reduced organic loading from sources not found in the GW to evaluate the ability of bioreactor consortia to utilize the GW contaminants as carbon sources.

* At this point, the bioreactors were considered acclimated.

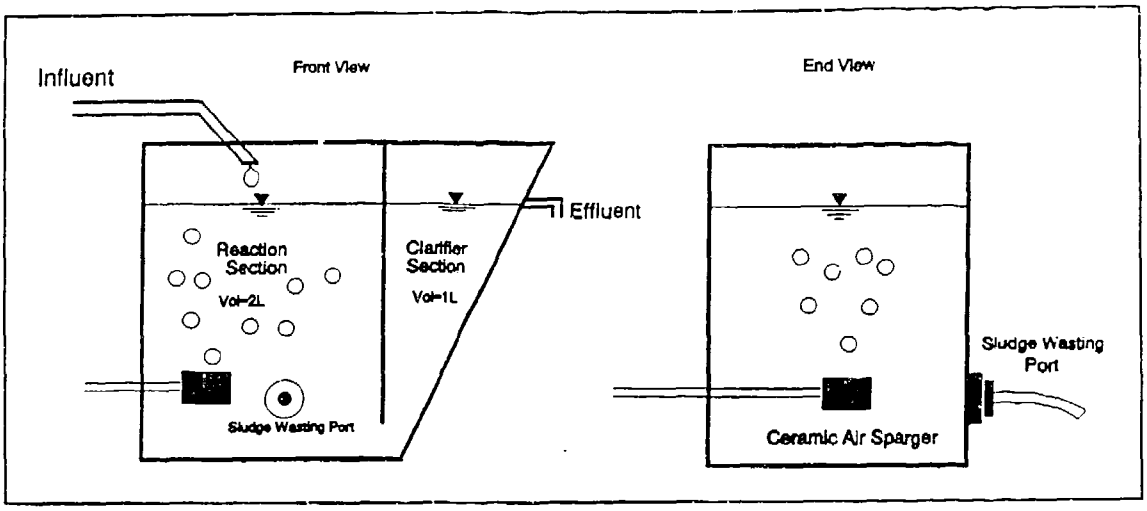



Figure 3. Bench-scale chemostat

Appendix A presents a chronological order of system operational changes and observations made throughout the course of this study. This information details some of the rationale of why various system operational changes were made.

The bioreactors have 2.0-ℓ aeration chambers and 1.0-ℓ in-vessel clarifiers. Influent was fed into the bioreactors using a Masterflex brand multiple stage peristaltic pump (Cole Palmer Inc., Chicago, IL). Sludge wasting for these reactors is accomplished through the sludge wasting port located on the side of the bioreactor (Figure 3). However, for this study, wasting of the sludge was not required since the rate of biomass growth roughly approximated the endogenous decay rate and systems solids loss rate over the weir, thereby maintaining a relatively constant biomass concentration.

Air and/or pure oxygen, when added, were sparged into the reactor using Fisher brand ceramic stones placed on the aeration chamber bottom. Sparging of air not only supplies oxygen, but also provides a means of mixing the ML. An in-line regulatory rotameter (Cole-Palmer Inc.) was used to control and measure flow rate. Bioreactor temperatures were maintained between 18 and 27 °C by controlling the laboratory temperature via the room air conditioning. Bioreactor influent and bioreactor ML did not require pH adjustments because the pH never was less than 6.5 or higher than 8.5.

## Microbial Seed Source

Bacterial inoculum was obtained from the City of Jackson, Mississippi, Sewage Treatment System (POTW), which is a 30-Mgd contact stabilization system. The City of Vicksburg uses an attached growth unit (trickling filter) for sewerage treatment and, therefore, was not considered a good microbial

seed source for suspended growth bioreactors. Inoculum samples were collected from the aeration tanks of the Jackson system after contact staging.

## Acclimation of Bacterial Consortia

Acclimation of microorganisms was accomplished by slowly introducing the microorganisms in the ML to the groundwater contaminants. Acclimation involves conversion of the microbial carbon food source (substrate) from the previous carbon source, the organics in municipal sewerage, to the groundwater contaminants, primarily benzene and added amendments.

The approach used in this study to acclimate the seed from the Jackson wastewater plant was to slowly convert composition of the system influent from 100-percent VPOTW wastewater to 100-percent RMA groundwater over a 2- to 3-month period (Table 1). The rate of influent composition conversion was based on close observation of any change in MLSS and MLVSS, BOD and COD removal, and SVI. Acclimation was considered complete when MLSS, MLVSS, gross pollutant removals (COD and BOD), benzene removal, and SVI had stabilized for greater than two SRTs.

Once the system influent consisted of only groundwater and the percent removal of gross pollutants and solid concentrations was stable, the consortium was considered acclimated. Acclimation of the bacterial consortium was considered complete after 4 months of operation. Sodium acetate and the sugars were added to increase the organic loading on the bioreactors, thereby maintaining a higher MLSS and MLVSS.

This approach is typical of most AS studies (Zappi and Morgan 1992 Zappi et al. (1990) and Zappi, Teeter, and Francingues (1990) successfully acclimated an AS consortia to a highly contaminated groundwater sample within a period of 10 weeks. Kim and Maier (1986) evaluated the acclimation and biodegradation potential of chlorinated organic compounds in the presence of cometabolites. They were able to acclimate a consortium of bacteria from a municipal AS plant capable of degrading 2,4-D (2,4 dichlorophenoxyacetic acid) and 3,5-DCB (3,5 dichlorobenzoate) under aerobic conditions. Combined contaminant concentrations as high as 100 mg/ℓ were successfully degraded. They concluded that seed bacterial consortia should contain as diverse a population of microorganisms as possible to increase the probability of plasmid exchange and that the acclimation phase should began with an influent containing very low concentrations of the target compounds to avoid inhibitory effects.

## Bioreactor Hydraulic Residence Times (HRTs)

During acclimation, the bioreactors were initially operated at an HRT of 5.6 hr (Table 1). As stated in Table 1, as the percentage of groundwater composing the influent increased, problems with poor sludge settling occurred. A clarifier is a phase separation process unit. The controlling factor to the overall performance of this process is the ability of the sludge to settle under reasonably quiescent conditions. Sludge with poor settling characteristics (measured as SVI) will not settle in clarifiers because of the lifting current associated with the hydraulic flux of effluent exiting the clarifier. Significant loss of solids from the clarifier is known as system washout. Washout can be associated with solids loss from the aeration chamber to the clarifier or, as is the case with this study, loss of solids from the complete biological system. Hydraulic flux in a clarifier is commonly referred to as surface overflow rate (SOR) and typically has the units of gpm/clarifier horizontal cross-sectional area. The HRT was reduced to minimize the potential for significant system washout by reducing SOR. As HRT increased, the dosing amount of cometabolites and nutrients was increased (Table 1) to maintain sufficient biomass activity.

From Table 1, when the system influent was composed completely of groundwater composite, the bioreactors were operated at an HRT of 17 hr. This value is consistent with the HRTs of other bioreactor systems treating contaminated groundwaters (Zappi and Morgan 1993).

## Sludge Retention Time (SRT)

Since sludge wasting was not performed, the SRT of the system is essentially indefinite. However, periodic loss of solids from the in-line clarifiers were observed indicating that there was a variable SRT associated with the bioreactors. Variability of SRT was based on the occasional solids losses via the exiting effluent because of periodic partial system washouts and endogenous decay. Since the solids loss over the bioreactor weirs were not quantified on a daily basis, then the actual bioreactor SRTs were not calculated.

## Influent Substrate and Nutrient Levels

Nutrients are required by all living organisms for synthesis of new cells, with nitrogen and phosphorus being the two major nutrients most often limiting to microbial activity. These nutrients are commonly referred to as macronutrients. Other inorganic compounds, referred to as micronutrients, are also required by microbes; however, the demand for these are low compared with that of the macronutrients. Most groundwaters undergoing biotreatment only require the addition of the macronutrients because sufficient amounts of micronutrients are usually naturally present. A widely recognized and accepted

approximation for appropriate nutrient levels is a carbon:nitrogen:phosphate (C:N:P) ratio of 100:10:1. During biotreatment of any wastewater or groundwater, ecological conditions established within the bioreactor should be maintained so that the only chemical limiting to further biological activity is organic carbon. To ensure this condition exists, dosing of nutrients into the influent at a C:N:P ratio of 100:10:1 is a good starting point; however, chemical analysis of the bioreactor effluent for nutrient levels should verify that residual nutrient levels are present. N and P were dosed into the influent to maintain effluent concentrations of at least 1 mg/ℓ of ortho-phosphate and 10 mg/ℓ of ammonia-nitrogen (however, on some days lower concentrations of N and P were observed and the influent adjusted accordingly). Table 1 lists the amendments added to influent throughout this study.

The influent from the VPOTW had an approximate BOD of 70 mg/ℓ; therefore, glucose and sodium acetate were added to the influent to increase the influent BOD to approximately 200 mg/ℓ during the early stages of acclimation when the influent was primarily composed of VPOTW influent. The amending of the influent continued as the influent became composed of primarily groundwater. From Table 1, the extent of cometabolite amending was increased when the influent was 100-percent groundwater because of the high HRT required to prevent solids washout. Amending with higher levels of cometabolites also ensured that the influent was able to support appropriate microbial activity within the three bioreactors. An MLSS of at least 1,000 mg/ℓ was targeted as a solids concentration that was considered indicative of sufficient microbial activity.

Admittedly, more optimization of bioreactor performance in terms of reduced cometabolite dose and groundwater HRT could have been made. However, the objective of this study was to evaluate if hydrogen peroxide could be used as an alternative oxygen source, not to evaluate the extent of bioreactor amendment optimization obtainable. The influent composition or bioreactor efficiency was not of prime concern. All that was required from the bioreactors for this study were biological systems treating an influent containing high levels of a regulated VOC that was being stripped from the bioreactor during aeration.

## Comparison of Alternate Oxygen Sources

The primary focus of this study was to evaluate the feasibility of using hydrogen peroxide as an alternative source of oxygen for aerobic, suspended growth bioreactors. As stated earlier, AS systems have traditionally used two sources of oxygen: air and pure oxygen. After acclimation of the microbial consortia to the benzene in the groundwater was complete, two of the three bioreactors, Nos. 1 and 2, were supplied air and pure oxygen as oxygen sources, respectively. These two bioreactors served as experimental controls representative of current technology. The performance of the hydrogen

peroxide fed, Bioreactor 3, was compared with Bioreactors 1 and 2. The comparison was based on the following criteria:

*a.* Contaminant removal.

*b.* VOC off-gassing.

*c.* Biomass stability.

*d.* Economics.

This comparison was performed once all three bioreactors had stabilized for 1 month. The air- and pure oxygen-sparged rates into the bioreactors were 1,145 and 73 ml/min, respectively. The source of the pure oxygen was bottled, reagent-grade oxygen purchased from Air Products Inc., Vicksburg, Mississippi. The flow rate of air or oxygen into the bioreactors was metered and measured using the in-line, regulatory rotameters.

## Hydrogen Peroxide Introduction

Bioreactor 3 was introduced to hydrogen peroxide beginning on Day 181. Introduction of the hydrogen peroxide into Bioreactor 3 was performed by slowly introducing small quantities of hydrogen peroxide into the reactor while full air sparging continued. The hydrogen peroxide was dosed into the bioreactors from a stock feed reservoir. The level of hydrogen peroxide in the feed reservoir was selected based on the flow rate of solution into the bioreactor and the targeted ML concentration. A bench-scale Master Flex brand 100-rpm-rated peristaltic pump was used to meter the stock hydrogen peroxide solution into Bioreactor 3.

Over time, the rate of air sparging into the bioreactor was reduced, while the amount of hydrogen peroxide added increased. Essentially, introduction of the hydrogen peroxide to Reactor 3 was viewed as another acclimation study phase. In this case, acclimation of the biomass to potentially toxic levels of hydrogen peroxide was required. Acclimation was evaluated based on stable solids concentrations and contaminant removals (BOD, COD, and benzene).

## ML Mixing

AS systems typically utilize aeration as a means of both providing oxygen and mixing the ML in the aeration chamber. Since hydrogen peroxide replaced aeration as an oxygen source, mechanical mixing was employed to maintain the ML in suspension. Lightnin brand Model TS2010 1/15-hp laboratory mixers were used to mix the ML. Paddle impellers were used to keep the ML mixed in the aeration chambers. The paddle impeller was selected because it did not induce mixing eddies within the in-line clarifier. When

difficulties associated with poor sludge settling were encountered, the paddle blades were slightly bent to cause an upward eddy to occur in the aeration chamber at the bottom of the baffle wall separating the aeration chamber from the clarifier. This adjustment improved the movement of settled sludge out of the clarifier back into the aeration chamber. All three mixers were set at 150 rpm.

The contents of all three bioreactors were mixed using the Lightnin mixers, even though the aerated bioreactor did not require mechanical agitation, so a direct comparison of VOC losses could be assessed. The oxygen-sparged bioreactor (No. 2) did require mechanical mixing because the low flow rate of oxygen into the bioreactor did not effectively keep the ML suspended. Photograph 1 shows the experimental setup used for this study.

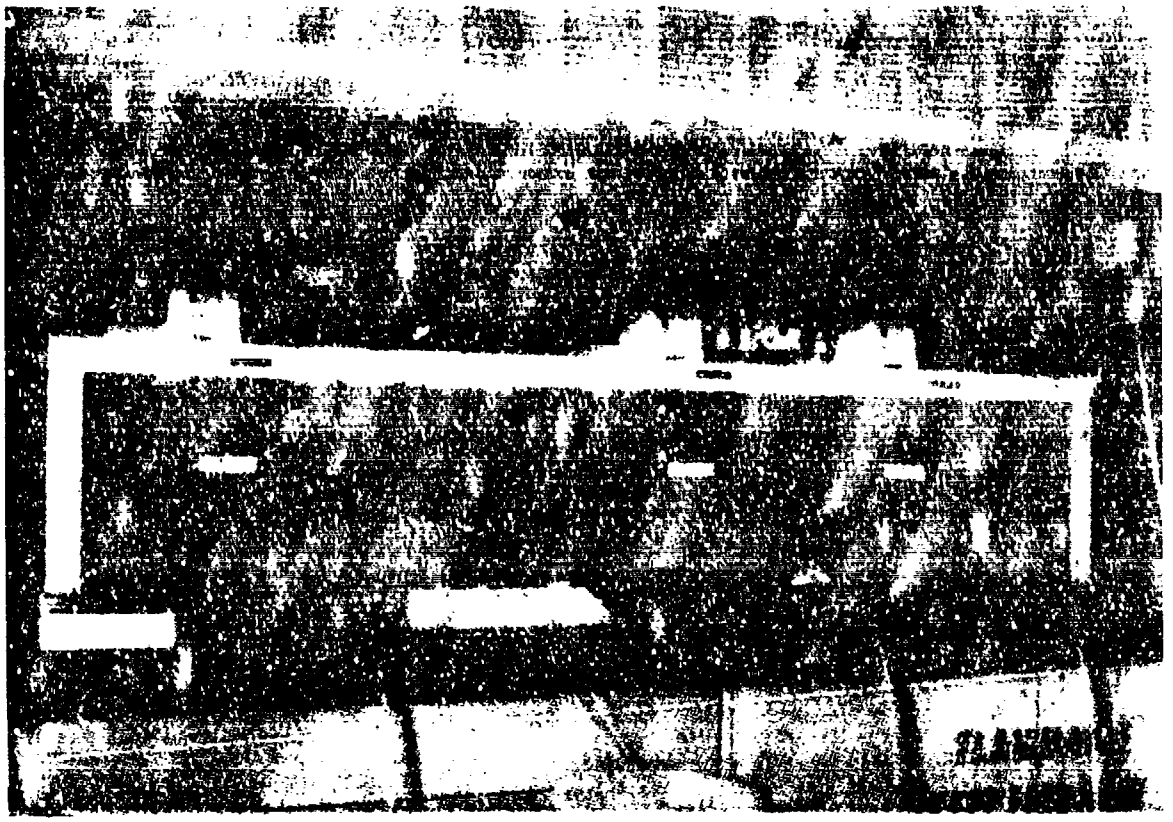
## Determination of Hydrogen Peroxide Fate

Several studies were conducted to evaluate the rate and determine kinetic data for the degradation of hydrogen peroxide, $H_2O_2$, by enzymatic pathways in the AS system. The AS samples used in these experiments were collected from chemostat-type reactors that were in operation within the WES Hazardous Waste Research Center in support of another ongoing study. These reactors have a volume of 10 ℓ and are fed VPOTW secondary influent. The reactors have MLSS and MLVSS concentration averaging 1,250 and 1,100 mg/ℓ, respectively. ML samples were not collected from the three bioreactors used in this study because of their limited volumes (2 ℓ) and concerns over potential adverse effects of these experiments on the ML.

For each experiment, a 1-ℓ volume sample of ML was collected from the reactors, placed in volumetric flasks, and stirred using a magnetic stir bar and plate. Hydrogen peroxide was batch dosed to the ML at the target levels and the residual hydrogen peroxide levels measured over time using the EM Quant strips and reflectometer. For some experiments, the ML was diluted using distilled water to achieve lower MLSS levels. Hydrogen peroxide concentrations were measured at various time increments and recorded. Any observation noted during the course of these experiments in terms of ML consistency or color was also recorded.

## Analytical Methods

The analytical techniques used in this study were performed by the technicians of the WES Hazardous Waste Research Center or the Environmental Chemistry Branch, WES. The methods and instruments used for these analyses are discussed below. The experimental methods used were obtained from *Standard Methods for the Examination of Water and Wastewater* (Greenberg, Clesceri, and Eaton 1992) unless otherwise noted.



Photograph 1. Experimental setup including mixers

## Catalase assays

Catalase degrades hydrogen peroxide to water and oxygen according to the following equation:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

In an attempt to understand the mechanisms and quantify the rates of hydrogen peroxide degradation in biological activated sludge reactors, quantification of catalase presence within each of the bioreactors was performed using a catalase assay technique. It was speculated that analysis of the ML for catalase would indicate greater amounts of catalase present in the bioreactor (Bioreactor 3) exposed to hydrogen peroxide than in the other two bioreactors. This speculation was based on the assumption that catalase could be detected in an ML matrix that contains both active and inactive cells. Hopefully, some of the inactive or dead cells were lysed, thereby releasing its cytoplasm, containing catalase, into the ML slurry.

The assay selected for catalase quantification was a modification of an assay obtained from Solvay Enzymes, Inc. Information about this assay was supplied by Mr. John Humphreys from Solvay Enzymes, Inc., Research and

Development, 1230 Rudolph Street, Elkhart, IN, 41514. This catalase assay was modeled after a method used by Beers and Sizer (1952) and modified by Chance and Machly (1955).

The method depends on spectrophotometrically tracking the enzymatic activity during degradation of a preset amount of hydrogen peroxide to water and oxygen at a wavelength of 240 nm. The assay defines a unit of enzymatic activity as the decomposition of $10^{-6}$ moles of hydrogen peroxide at specified conditions at 25 °C. This assay uses bovine liver catalase to degrade hydrogen peroxide under a timed, sequenced spectrophotometric analysis to determine catalase activity.

A stock solution of catalase enzyme was made by dissolving 0.1 g of the solid lyophilized enzyme in 50.0 ml of 0.005 M phosphate buffer that maintained a pH of 7.0 (~1:500). This solution was labeled as Solution A and was used as stock solution for catalase. A second solution, Solution B, was formulated by diluting 0.1 ml of Solution A to 10 ml with deionized distilled water (~1:50,000). Continual serial dilutions were made to test the sensitivity of the catalase assay.

A hydrogen peroxide solution was made by diluting 0.3 ml of 30-percent $H_2O_2$ to approximately 0.059 M by adding it to 50 ml of 0.05 M phosphate buffer (pH = 7.0). Several serial dilutions of known concentrations of hydrogen peroxide were made either by dilution of 50 or 30-percent hydrogen peroxide by weight to the appropriate concentration.

A 0.05 M phosphate buffer, used as the baseline reagent matrix, was made by making a solution of 0.1 M phosphate buffer (pH 7.0), then by diluting 1:1 with distilled water. A ratio of 50 ml of 0.1 M $KH_2PO_4$ (13.56 g/ℓ) was mixed with 29.1 ml of 0.1 M NaOH (4.0 g/ℓ). Doubling the volume of the final mixture with distilled $H_2O$ gave a phosphate buffer of 0.05 M (pH 7.0). It can be noted that this assay can be adapted for measurement of catalase activity by making serial dilutions of catalase in the presence of excess hydrogen peroxide, or it can be used for measurement of hydrogen peroxide by producing a standard curve of hydrogen peroxide by serial dilutions in excess of catalase enzyme.

Before modification, the assay was first run to become familiar with the procedures. Solutions were prepared as specified in the assay, and the procedure was run successfully several times. The change in absorbance (A) versus time was measured, and a value for catalase activity was obtained. An example of the change in absorbance versus time for the assay is shown at Figure 4. Samples were added directly to a fused silica sample cuvette (3-ml volume and 1.0-cm path length) by first introducing 2.0 ml of enzyme, then by adding 1.0 ml of hydrogen peroxide substrate. The reference cell contains 2.0 ml of water and 1.0 ml of hydrogen peroxide. Readings were taken at time zero and then at 10-sec intervals. The theoretical absorbance at time zero for a 0.059 M concentration of hydrogen peroxide is expected to be about 0.850. Constant

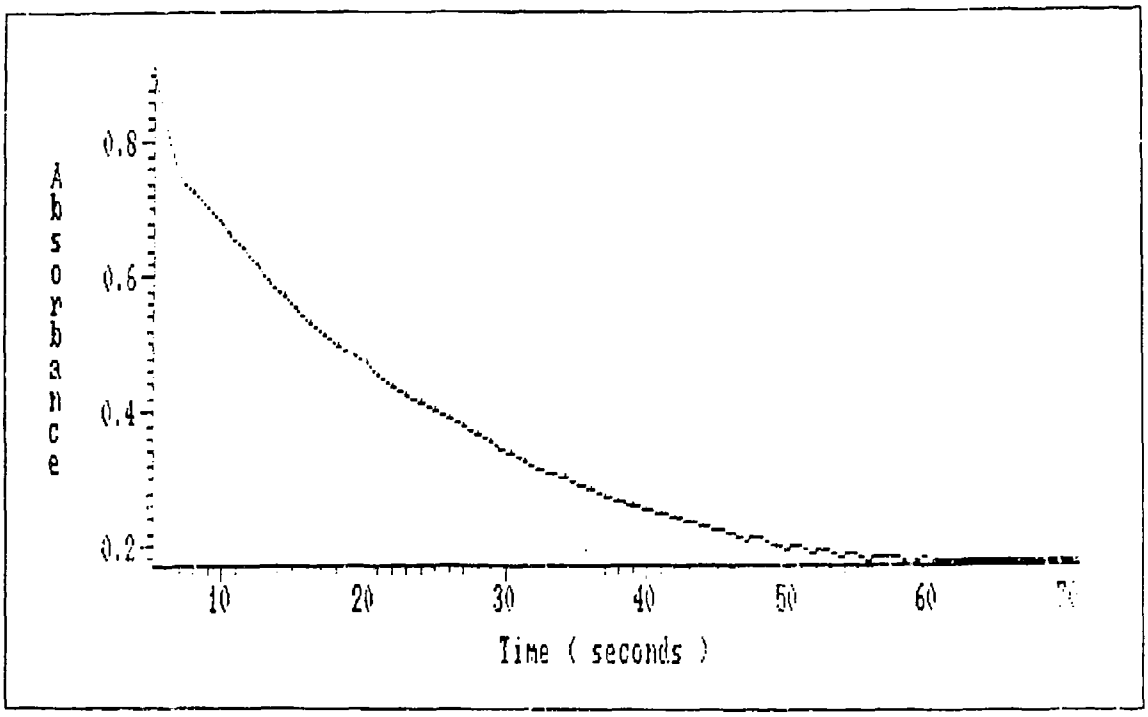



Figure 4. Spectrophotometric trace of enzyme absorbance

agitation to remove oxygen bubbles formed by peroxide degradation was necessary.

A spectrophotometer with computer interface was used to measure absorbance. A program was developed for the spectrophotometer that monitored the loss of hydrogen peroxide by measuring changes in absorbance at 240 nm. Absorbance was monitored over a 70-sec time period, with readings taken every 10 sec. Data generated was stored on computer disk, and hard copy graphs of absorbance versus time were produced.

To conduct the test, 2 ml of the enzyme were added to the cuvette and placed in the spectrophotometer. One milliliter of the hydrogen peroxide solution was then pipetted directly into the cuvette. The test program of the computer was started simultaneously with the addition of the hydrogen peroxide, and the change in absorbance was monitored for 70 sec. The change in absorbance was calculated, and a resulting enzymatic activity level was computed as units/milligram or units/milliliter of sample (method of calculation shown below). It can be noted from Figure 4 that absorbance started at about 0.9 and decreased rapidly during the test to a minimum value.

After familiarization with the instrument and assay, the procedures were adapted to examine the water from Bioreactor 3, which used hydrogen peroxide as an oxygen source. To test for enzymatic activity in Bioreactor 3, the enzyme solution specified in the assay was replaced with filtered water from the reactor. An aliquot of water from Bioreactor 3 was obtained and then

filtered to remove solids. The filtrate was then filtered two times through a 0.45-µm Millipore filter to remove any remaining particulates. Several concentrations of hydrogen peroxide solutions were prepared, in addition to those specified by the assay.

The method of calculation used was the same as described by Solvay Enzymes, Inc. The catalytic activity is given as units per milliliter, or units per milligram of sample. A unit is given as 1 µmol of hydrogen peroxide decomposed per minute at 25 °C. The change in absorbance per minute, ΔA/min, was determined by subtracting the final absorbance from the initial absorbance, and the catalase activity was calculated using the equation given in the assay, shown below:

$$\text{units/mg} = \frac{\Delta A/\text{min}}{\varepsilon \times \text{mg catalase/ml reaction mixture}}$$

where

$A$ = absorbance

$\varepsilon$ = molar absorptivity, ($A \times 1 \times \text{cm}^{-1} \times \text{mol}^{-1}$)

units = µmol $H_2O_2$ decomposed/min at 25 °C

## Solids

Method Nos. 2540D and 2540E were used for determining MLSS and MLVSS, respectively. A Cole-Palmer brand convection oven and Thermochem brand muffle furnace were used for sample drying. A Mettler brand four place analytical balance was used for weighing the samples. Millipore brand 0.45-µm glass fiber filters were used with Millipore all-glass filter systems for dewatering the ML.

## Sludge volume index (SVI)

SVI was determined following Method 2710D with one minor modification. That modification was that a 100-ml graduated cylinder was used instead of the recommended 1,000-ml graduated cylinder. The smaller cylinder was used because of the small volume of the chemostats used in this study.

## Temperature and pH

An Orion Model EA940 pH meter and probe was used for analyzing pH. Fisher brand thermometers were used during temperature measurements.

### Dissolved oxygen (DO)

DO Method 4500 OG was followed using an Orion Oxygen Analyzer Model 840 and probe to measure DO. Air calibration technique was used for system calibration.

### Oxygen uptake rate (OUR)

Method 2710B was used for determining ML OUR using an Orion Model 840 oxygen analyzer and probe. Air calibration technique was used for meter and probe calibration.

### Biochemical oxygen demand (BOD) and chemical oxygen demand (COD)

BOD test methods used in this study were Method 507, which utilizes a DO meter and probe. The COD was measured as described in Method 5220D, which used a Hach digester and colorimeter.

### Hydrogen peroxide

A variety of analytical techniques for hydrogen peroxide were evaluated during this study. The reasons for evaluating several methods was that the poor light transmittance, complex chemical matrix, and rapid reactions associated with the bioreactor ML made analysis of hydrogen peroxide using traditional techniques difficult. Appendix B details the evaluation efforts made during this study to select an appropriate method. Based on these activities, an RQFlex Reflectometer (EM Science Inc., Gibbstown, NJ) was used to measure hydrogen peroxide levels. This system uses a colorimetric type test to measure hydrogen peroxide residuals in the range of 0.2 to 20 mg/ℓ. Because of the limited measurement range of the instrument, the samples taken from the ML were diluted with distilled deionized (DDI) water. This was accomplished by diluting a 5-ml sample with 95 ml of DDI water for a 20:1 dilution. Results displayed on the meter were then multiplied by 20.

### VOC analysis

Liquid phase VOC analysis was performed using a Hewlett Packard Model 5890 gas chromatography unit and OI brand purge and trap sample concentrator. The VOC method used was U.S. Environmental Protection Agency (USEPA) Method 602.

Air phase VOC analysis was performed using an HNU Inc. Model 101 photoionizer volatile organic compound analyzer that was calibrated specifically for direct benzene concentration output.

# 3 Study Results

## Acclimation Phase

The Acclimation Phase of the study was performed to acclimate a microbial consortia to the high levels of benzene in the amended groundwater influent. Various aspects of the Acclimation Phase are discussed below. In all of the figures used to present data from this study, Day 1 represents 3 weeks of bioreactor operation prior to any addition of groundwater to the influent. The period of time prior to Day 1 is not of importance because it was used to fine-tune bioreactor operations and focused on operation of the bioreactors using VPOTW influent. One point of interest on the study activities prior to Day 1 is that a sequential batch reactor (SBR) system was originally used for maintenance of the biomass, but poor sludge settling resulted in significant solids loss during the decant stage. Conversion to AS resulted in improved solids capture because of improved sludge recirculation using the in-vessel clarifiers.

### SVI

Figure 5 presents the SVI values measured during the Acclimation Phase for all three bioreactors. As expected, during the period when the influent to the bioreactors was composed of primarily POTW influent, the SVIs fluctuated around the 100 value (an SVI of 100 is considered characteristic of a sludge with excellent settling characteristics). However, as the influent groundwater composition increased, the SVIs increased to levels in excess of 1,000 at times. Sludge containing microbial consortia undergoing significant physiological changes, such as new members of the consortia flourishing and populations of previously dominant members decreasing, typically has characteristically high SVIs. This speculation is further substantiated by the low solids concentrations and high MLVSS to MLSS ratios observed (these data are presented next).

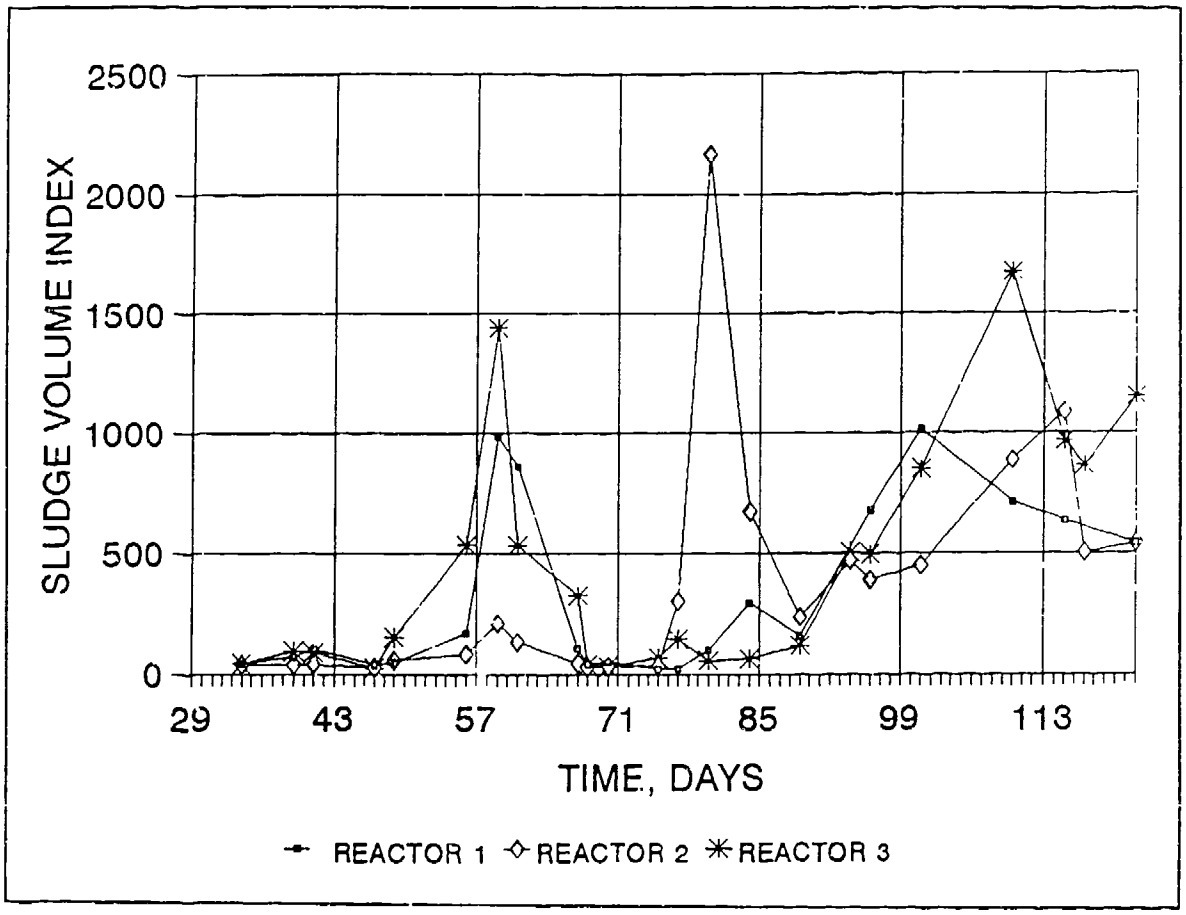



Figure 5. Acclimation Phase SVI data

## MLSS

Figure 6 presents the MLSS data for the three bioreactors during the Acclimation Phase. At the initiation of the Acclimation Phase prior to any groundwater addition (Days 12 through 26), a washout of ML biomass occurred because of technician error in terms of setting the influent pump flow rate. As a result of the washout, additional ML from the Jackson POTW was added to the aeration chambers of all three bioreactors to increase the solids concentration in the aeration chamber. This activity resulted in the elevated MLSS concentrations measured on Day 28. As the portion of groundwater added to the influent increased, MLSS levels in all three bioreactors decreased and eventually stabilized at approximately 1,000 mg/ℓ when the influent was composed of 100-percent amended RMA groundwater.

## MLVSS

Figure 7 presents the MLVSS data for the three bioreactors during the Acclimation Phase. The MLVSS data generally followed the same trend

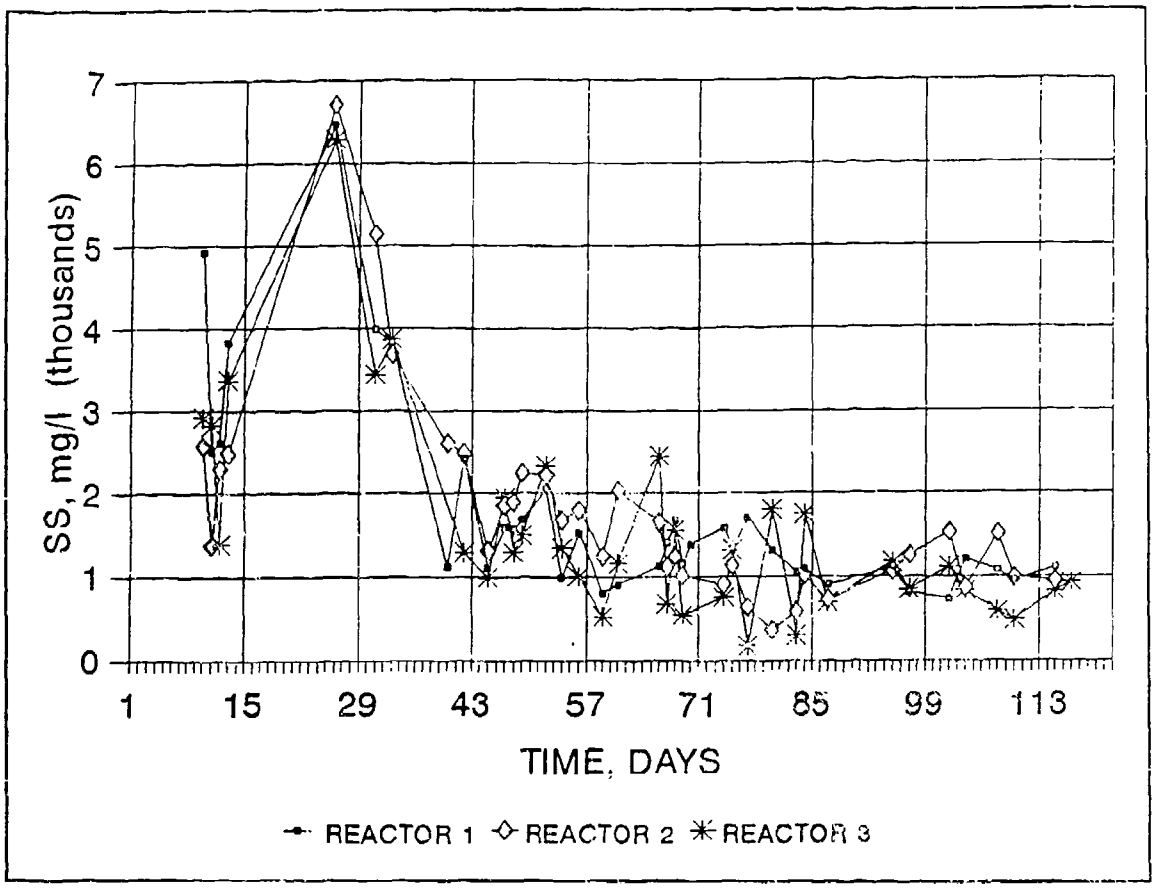



Figure 6. Acclimation Phase MLSS data

observed with the MLSS data (Figure 6). The ratios of MLVSS to MLSS over the course of the Acclimation Phase were greater than 80 percent, indicating an ML with a low fixed solids content and high active biomass concentration. These ratios are indicative of a dynamic sludge that appears to be undergoing possible physiological changes as the influent substrates became more complex in terms of chemical composition.

## BOD removal

Figure 8 presents the influent and effluent BOD for all three bioreactors measured during the Acclimation Phase. As the amount of groundwater composing the influent increased, influent BOD also increased. The bioreactors did not indicate any adverse effects on BOD removal as the levels of benzene present in the influent increased (this was due to the increased amounts of RMA groundwater composing the influent). BOD removals in excess of 95 percent were maintained throughout the Acclimation Phase, indicating a stable bioreactor system. There was little observed difference in bioreactor performance towards BOD removal.

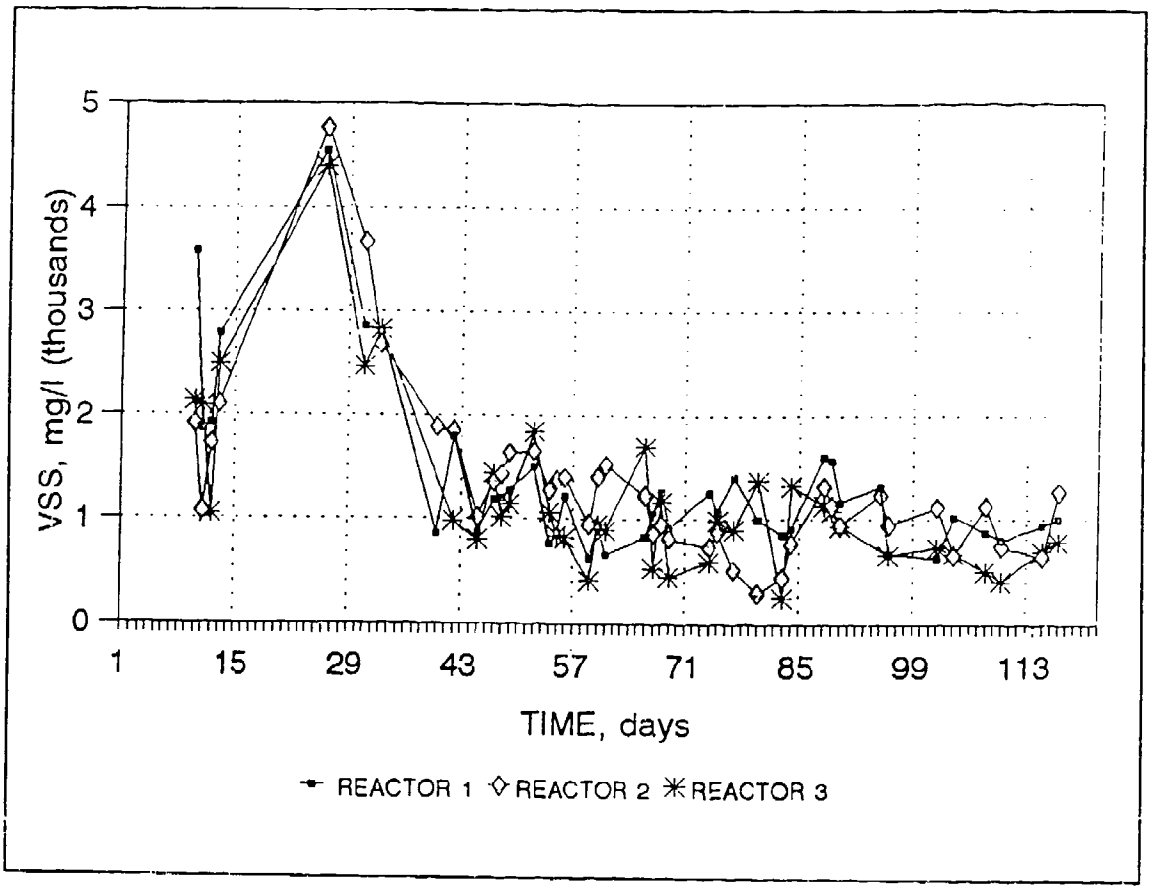



Figure 7. Acclimation Phase MLVSS data

## COD removal

Figure 9 presents the COD concentrations analyzed in the influent and effluents for all three bioreactors during the Acclimation Phase. From the figure, approximately 50-percent removal of COD was achieved within all three bioreactors. As the composition of the influent increased in terms of groundwater content, the effluent CODs increased. When comparing the BOD data (Figure 8) to the COD data (Figure 9), it is apparent that the groundwater contained an organic fraction that was not easily oxidized by the microbial consortia in the bioreactors. By Day 113, when the influent was 100-percent groundwater, the effluent CODs from all three bioreactors had increased from levels below 100 mg/ℓ when no groundwater was present to levels centering around 300 mg/ℓ. The data also show that the bioreactors generally performed equally in terms of COD removal.

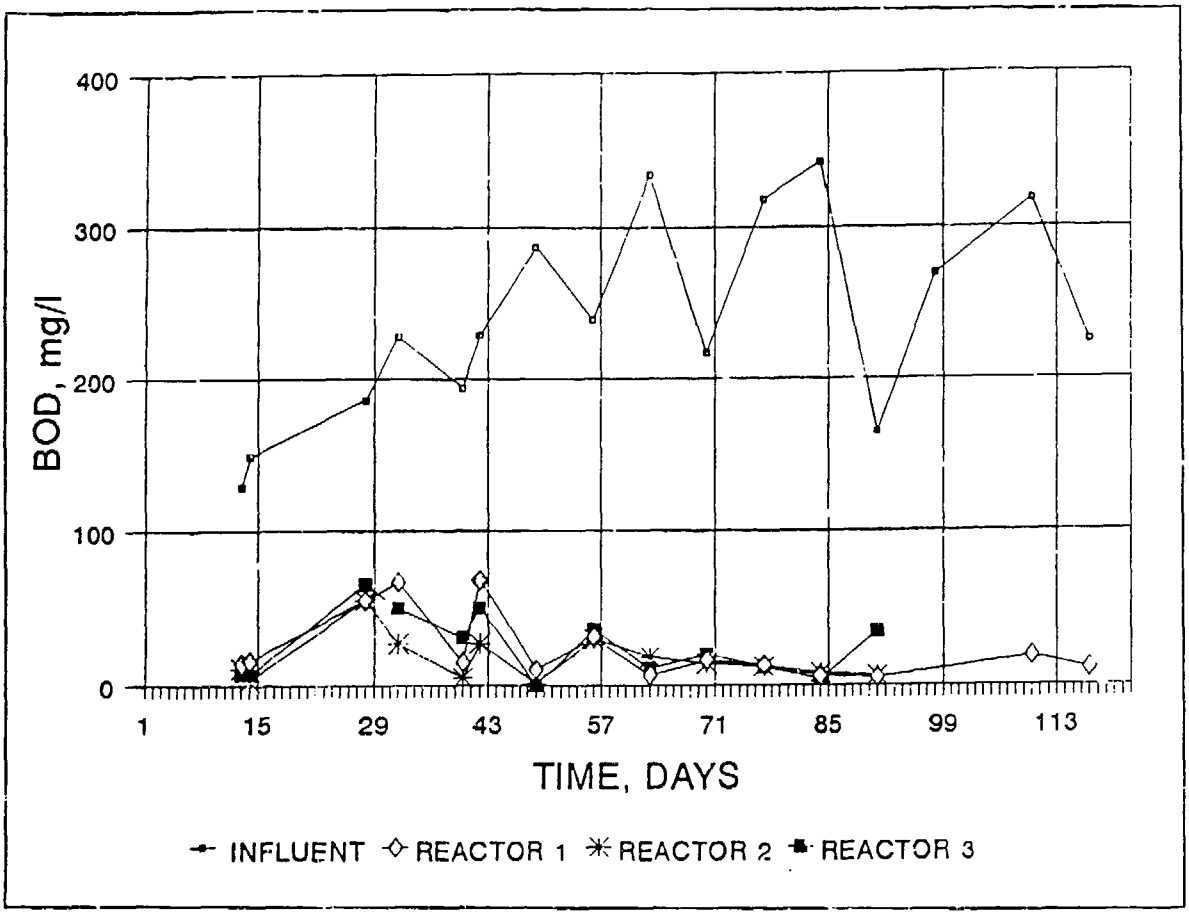



Figure 8. Acclimation Phase BOD data

## Benzene

Figure 2 presents the total benzene data versus test time for the bioreactor influent throughout the complete study. The POTW influent, as expected, did not contain measurable amounts of benzene. Therefore, as the percentage of groundwater composing the influent increased, so did the measured levels of benzene in the influent increase. Benzene was rarely detected in the effluents from any of the bioreactors during the Acclimation Phase of study, indicating almost 100-percent removal of the benzene (which is why effluent benzene levels were not plotted). These data indicate a high potential for removing benzene from the RMA groundwater using biological processes.

## Extent of acclimation

Figure 10 presents the results of an evaluation of the extent of acclimation achieved using respirometric techniques. During these experiments, systems influent was loaded into four microcosms seeded with nonacclimated biomass from the Jackson POTW and four microcosms containing equal portions of

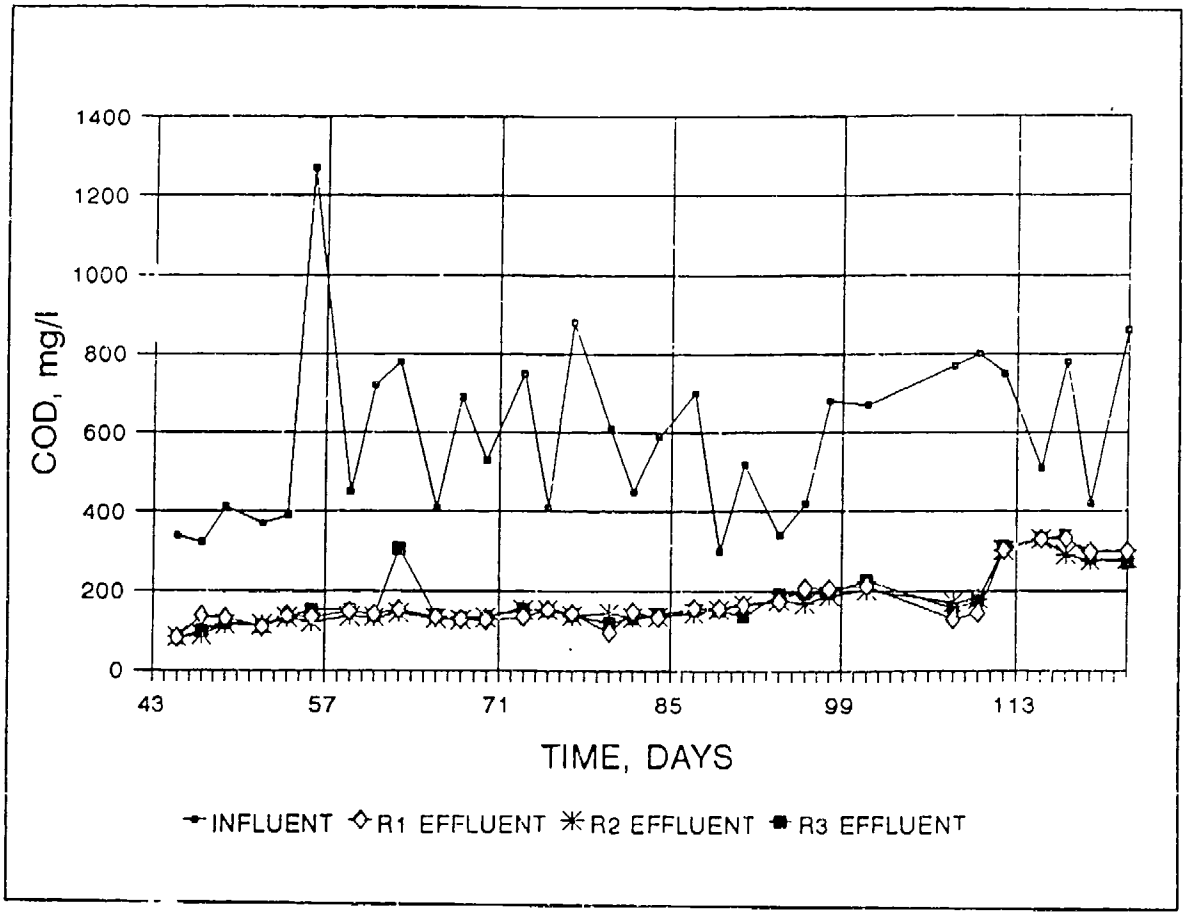



Figure 9. A climation Phase COD data

acclimated b:( ıass from the three study bioreactors. These data indicate that the overall o; ; ;en utilized over time was consistently greater throughout the 70 hr of testi , . Clearly, the microbial consortia were much more efficient in terms of carb substrate usage.

## Summary

The consortia was considered acclimated by Day 113 based on the stability of the consortia in terms of contaminant removals, solids concentrations, and complete conversion of the influent to amended groundwater sample. The consortia did indicate evidence of slight inhibitory effects because of the chemical complexity of the groundwater sample and poor cell synthesis potential. At this point in the study, it was decided to proceed to the Hydrogen Peroxide Introduction Phase of study.

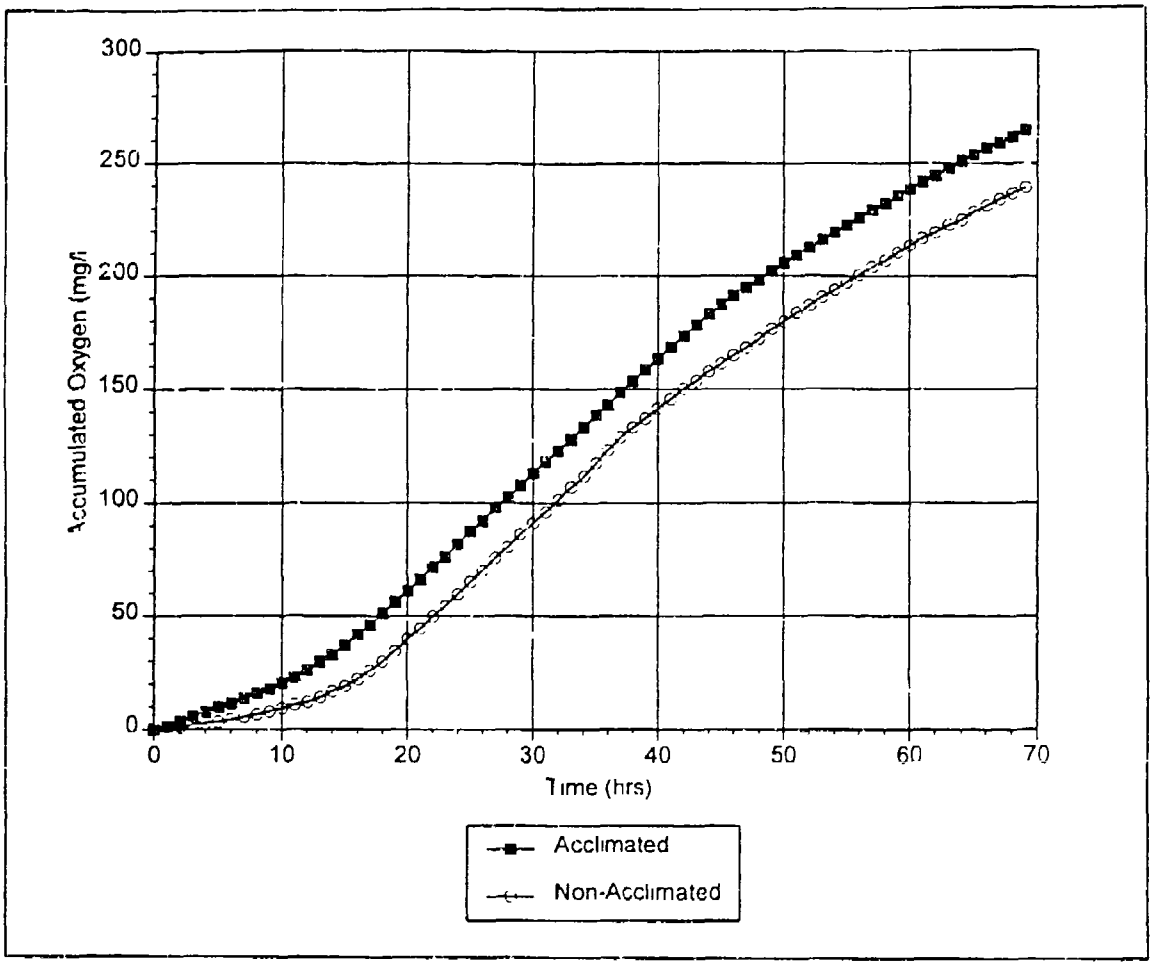



Figure 10. Comparison of acclimated versus unacclimated biomass toward $H_2O_2$ usage

## Hydrogen Peroxide Introduction

This phase of study was designed to evaluate the ability of AS to utilize hydrogen peroxide as the sole oxygen source. A variety of approaches to introduction of residual levels of hydrogen peroxide were attempted. The following discussion describes these efforts, then summarizes the results.

### Hydrogen peroxide acclimation

On Day 181, a 50-mg/ℓ hydrogen peroxide stock solution was introduced to Bioreactor 3 at a rate of 10 ml/hour. Mass balance of hydrogen peroxide around the bioreactor was developed as follows:

$$MASS_{IN} = MASS_{OUT} - ACCUMULATION - REACTION(S)$$

The above equation represents the basic mass balance equation for any closed system. For initial development of hydrogen peroxide feed rate estimates, accumulation and reaction terms were considered zero (i.e., hydrogen peroxide is a conservative species). It was realized when this assumption was made that significant reactions are likely to occur within the bioreactor. However, a nonreactive hydrogen peroxide dose was determined using the following equation

$$(C_{HP} \times Q_{HP}) + (C_{IN} \times Q_{IN}) = (C_{EF} \times Q_{EF})$$

where

$C_{HP}$ = concentration of $H_2O_2$ in the stock solution, mg/ℓ

$Q_{HP}$ = flow rate of stock solution into the bioreactor, ml/min

$C_{IN}$ = concentration of $H_2O_2$ in influent, mg/ℓ

$Q_{IN}$ = influent flow rate, ml/min

$C_{EF}$ = concentration of $H_2O_2$ in the bioreactor effluents, mg/ℓ

$Q_{EF} = Q_{IN} + Q_{HP}$, ml/min

Note that by definition of a completely mixed reactor, $C_{EF}$ is also the hydrogen peroxide concentration within the aeration chamber. Also, the hydrogen peroxide concentration in the system influent is equal to zero. Further manipulation of the nonreactive, mass balance equation yields

$$C_{EF} = \frac{Q_{HP} \times C_{HP}}{Q_{EF}}$$

This concentration is referred to as the applied residual hydrogen peroxide dose. Applied residual hydrogen peroxide dose is another method for describing hydrogen peroxide dose. The other method is mass of hydrogen peroxide dosed per unit time. The applied residual dose is the concentration of hydrogen peroxide that is calculated based on the amount of hydrogen peroxide fed into a complete-mix reactor with no reactions occurring involving hydrogen peroxide. In other words, hydrogen peroxide is treated like a nonreactive species. Using a stock solution of 50 mg/ℓ dosed at a 10-ml/min rate and assuming no reaction of the hydrogen peroxide within the aeration chamber, an applied residual hydrogen peroxide concentration of 4.17 mg/ℓ is estimated.

Aeration was continued in the bioreactor as hydrogen peroxide was initially introduced into the bioreactor. This was done to ensure sufficient aerobic conditions while allowing evaluation of the impact of the residual hydrogen peroxide levels on the biomass since hydrogen peroxide can be toxic to microorganisms (see Chapter 1). It was feared that localized pockets of the concentrated hydrogen peroxide stock solution at the point of injection into the bioreactor could damage the ML biomass. Periodically, the concentration and dosing rate of the hydrogen peroxide stock solution was increased to evaluate if any adverse impacts of injecting higner levels of stock solutions would be observed.

Table 2 summarizes the various combinations of hydrogen peroxide dosing schemes evaluated (i.e., stock feed flow rate and stock solution concentrations). The various doses of hydrogen peroxide evaluated did not appear to adversely impact the activity of the biomass based on visual observations and review of operational data (solids concentrations, contaminant removals, and nutrient usage).

**Table 2**
**Chronological Order of Events During Hydrogen Peroxide Introduction**

| Test Day Dose | Air Flow Rate cc/min | Stock $H_2O_2$ Solution mg/ℓ | $Q_{HP}$ cc/hr | Residual $H_2O_2$ mg/ℓ |
|---|---|---|---|---|
| 181 | 1,500 | 50 | 10 | 4.2 |
| 206 | 1,500 | 200 | 10 | 16.8 |
| 208 | 1,500 | 200 | 20 | 33.6 |
| 215 | 1,500 | 1,000 | 20 | 168 |
| 217 | 1,500 | 1,000 | 40 | 336 |
| 285 | 1,145 | 1,000 | 40 | 336 |
| 297 | 699 | 1,000 | 40 | 336 |
| 306 | 0 | 1,000 | 40 | 336 |

## Oxygen uptake rate (OUR)

Oxygen uptake rate experiments were performed to estimate the amount of molecular oxygen (in terms of dosed mass) that must be introduced into Bioreactor 3 via injection of the stock hydrogen peroxide. Figure 11 presents the OUR data for Bioreactors 1 and 3 that were generated by turning off airflow into the bioreactor and monitoring ML DO over time. These data indicate that the OUR for both bioreactors was approximately 0.32 mg DO/ℓ/min indicating that the injection of 50 mg/ℓ of hydrogen peroxide into Bioreactor 3 was not inhibitory nor significant enough to appreciably meet oxygen demand.

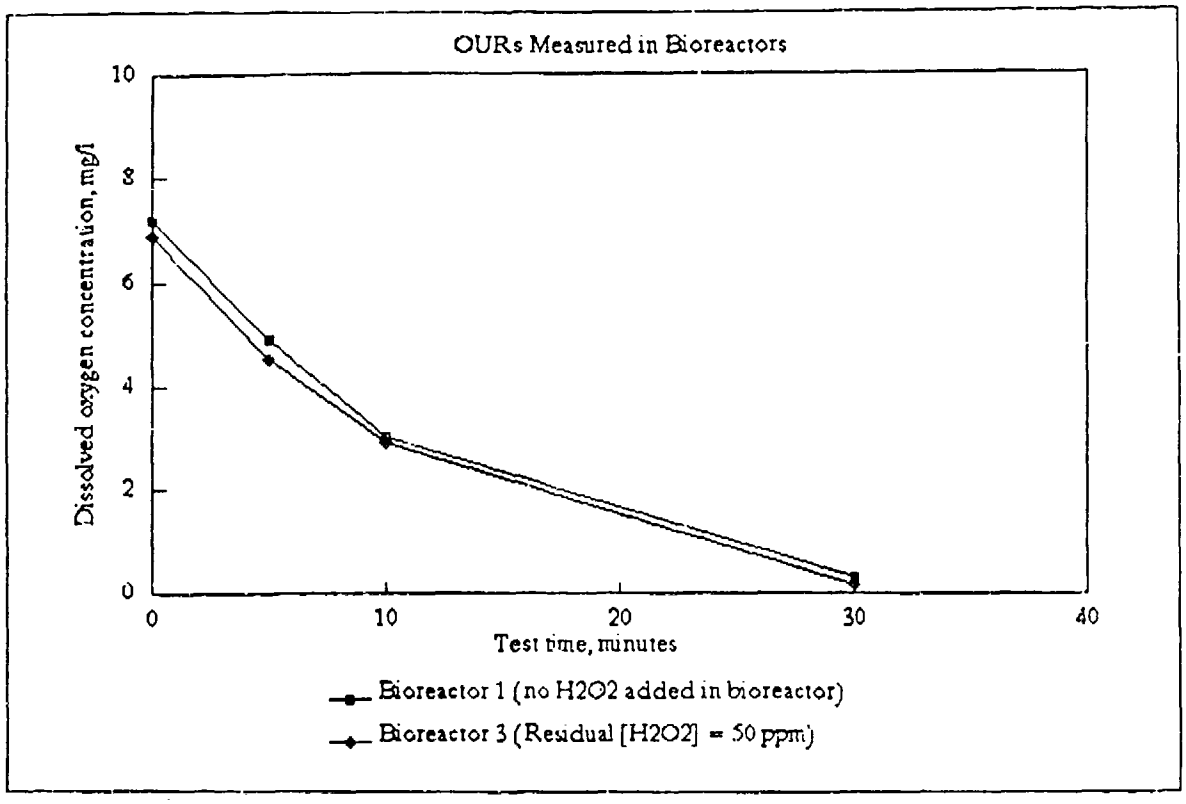



Figure 11. Bioreactor OUR data

Figure 12 presents the results of an experiment performed to compare the OUR of a bioreactor without previous hydrogen peroxide exposure with one with an acclimated microbial population. The resulting lower OUR in the unexposed reactor indicates that acclimation to hydrogen peroxide is required. The results of this experiment indicate that the bioreactor OUR for Bioreactors 1 and 3 were 0.056 and 0.053 mg DO/ℓ/min, respectively. The addition of 50 mg/ℓ hydrogen peroxide did reduce the OUR of the bioreactors by 67 percent. The reduction was on the same level for both reactors, indicating the acclimation of the microorganisms to residual hydrogen peroxide was not required. Also, the results indicate that hydrogen peroxide appeared to be a good oxygen source. It must be pointed out that another conclusion could have been drawn from these data. The lower OURs observed could have been attributable to reduced microbial activity caused by an inhibitory or toxic effect of the hydrogen peroxide on the microorganisms. However, this conclusion was not considered viable because of the low level of hydrogen peroxide dosed. As stated in Chapter 1, literature suggests that levels at least an order of magnitude or higher are required to adversely impact the activity of most microorganisms.

Hydrogen peroxide is approximately 98-percent molecular oxygen. However, hydrogen peroxide breakdown was assumed using the mechanism previously presented (Chapter 2).

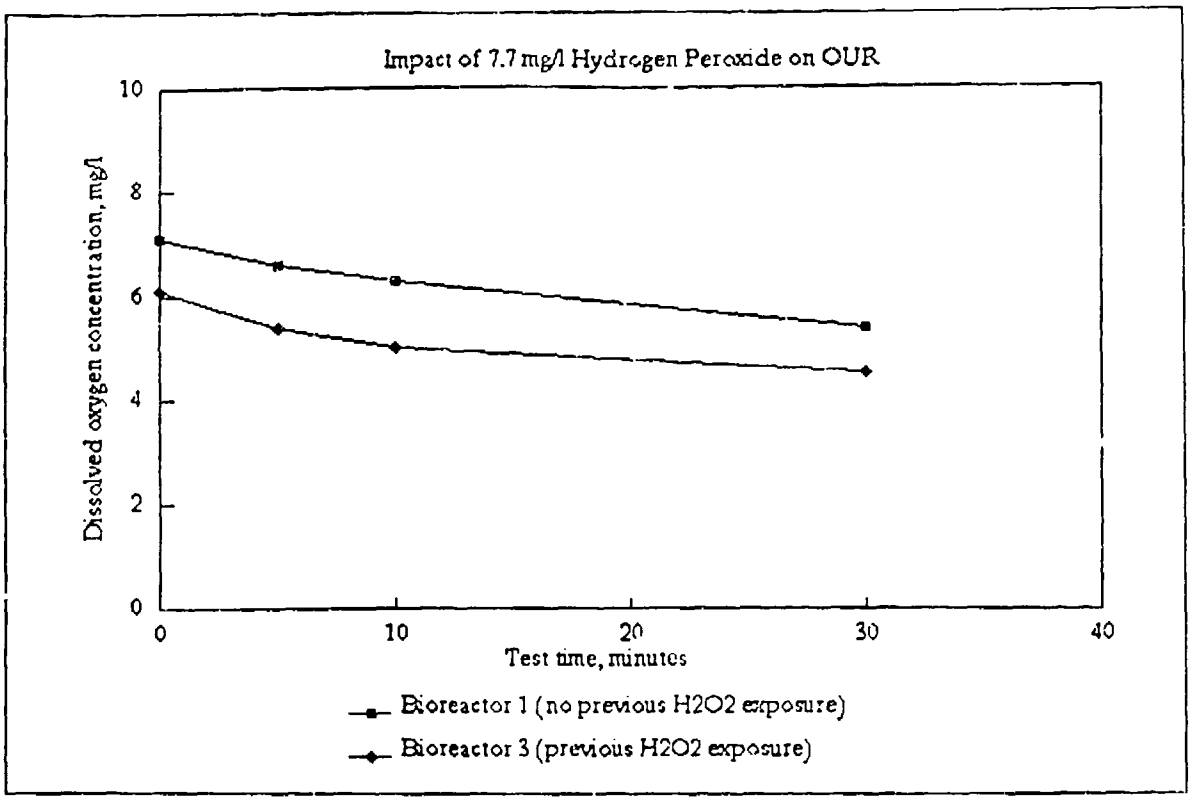



Figure 12. Comparison of acclimated biomass OUR to unacclimated biomass OUR

$$2H_2O_2 + \text{catalase} \rightarrow 2H_2O + O_2$$

Based on the above degradation reaction and to satisfy an OUR of 0.16 mg DO/ℓ, hydrogen peroxide must be dosed at a rate of 0.34 mg $H_2O_2$/liter ML/minute. The bioreactors used in this study had an effective volume of 2 ℓ requiring the addition of hydrogen peroxide into the bioreactor at a dosing rate of approximately 0.64 mg $H_2O_2$ per minute to satisfy the OUR of the ML consortia.

Figure 13 presents data from three experiments performed to evaluate the impact of various applied hydrogen peroxide doses on OUR. Figure 13 indicates that increasing the applied hydrogen peroxide dose into a bioreactor does reduce OUR based on changing oxygen levels over time. Applied dosages of 16.8 mg/ℓ and 33.6 mg/ℓ represent oxygen mass delivery rates of 0.034 $H_2O_2$ mg/min and 0.068 mg $H_2O_2$/min. These delivery rates were on an order of magnitude too low for meeting the measured OUR of bioreactors.

Based on the calculated OUR for the bioreactors, an applied residual hydrogen peroxide dose of 333 mg/ℓ was selected. A hydrogen peroxide stock solution of 1,000 mg/ℓ and dosing flow rate of 40 ml/hr were used to obtain this dose. These conditions represented maximum dosing conditions based on

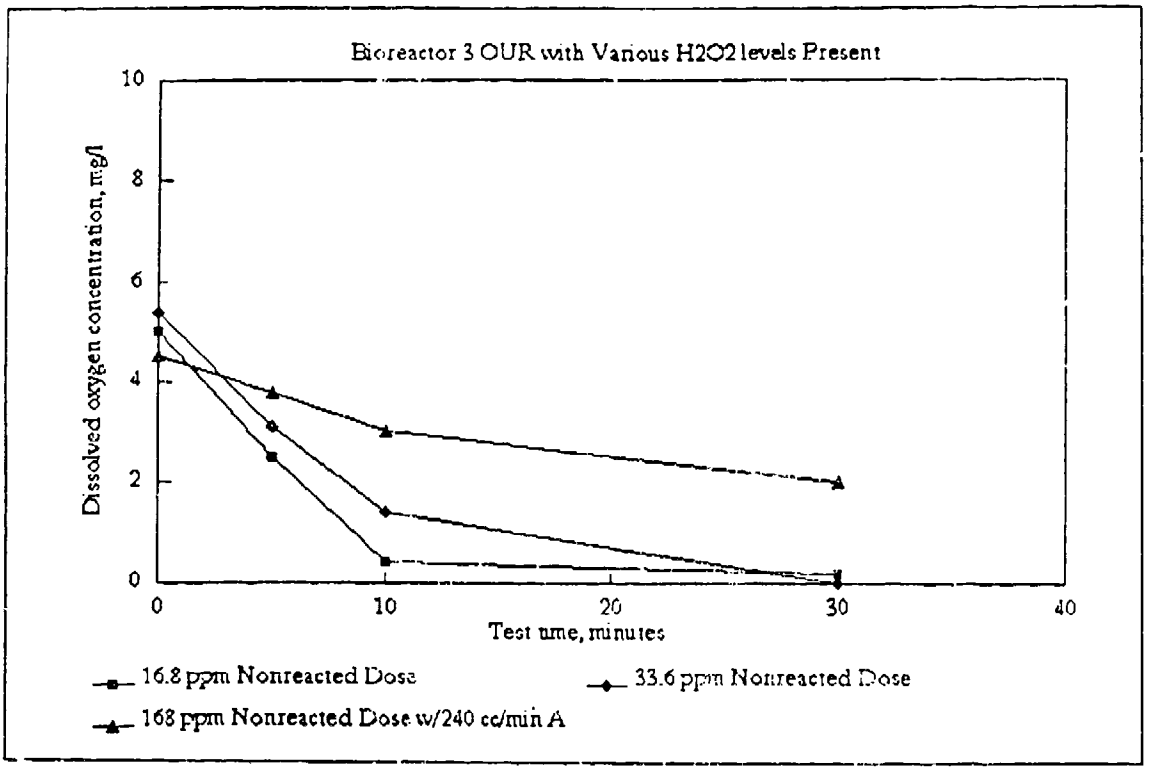



Figure 13. Evaluation of applied residual $H_2O_2$ dose on OUR

the assumption from literature that hydrogen peroxide levels in excess of 1,000 mg/ℓ would have a toxic effect on the ML biomass. Also, dosing flow rates greater than 40 ml/hr would represent greater than 35 percent of the total liquid fed into the bioreactor. This dosing represents an oxygen delivery rate of approximately 0.66 mg $H_2O_2$/min. This delivery rate (0.66 mg/min) was approximately equal to the OUR measured in Bioreactor 3 (0.32 mg DO/min).

## Conversion of Bioreactor 3 to hydrogen peroxide as an oxygen source

The conversion of Bioreactor 3 to hydrogen peroxide as the sole oxygen source was completed on Day 306. After the full conversion to hydrogen peroxide was complete, specific attention was paid to DO levels in the reactor. Extremely low levels of DO were detected, usually less than 0.5 mg/ℓ. Additionally, analysis for residual hydrogen peroxide levels in the reactor were performed using the EM Quant test strips, with hydrogen peroxide never being detected in the aeration chamber of Bioreactor 3. The low DO and residual level of hydrogen peroxide in the bioreactor were not unexpected. The hydrogen peroxide dosing rate was calculated and dosed to meet the OUR of the bioreactor. Residual levels of hydrogen peroxide or DO were not expected to be found in the bioreactor.

## Bioreactor performance during hydrogen peroxide introduction

Figures 14 and 15 present the COD and BOD influent and effluent data, respectively, for all three bioreactors while Bioreactor 3 was being introduced to the presence of residual hydrogen peroxide levels within the aeration chamber. These data indicate little or no adverse impact on bioreactor performance in terms of gross pollutant removal.

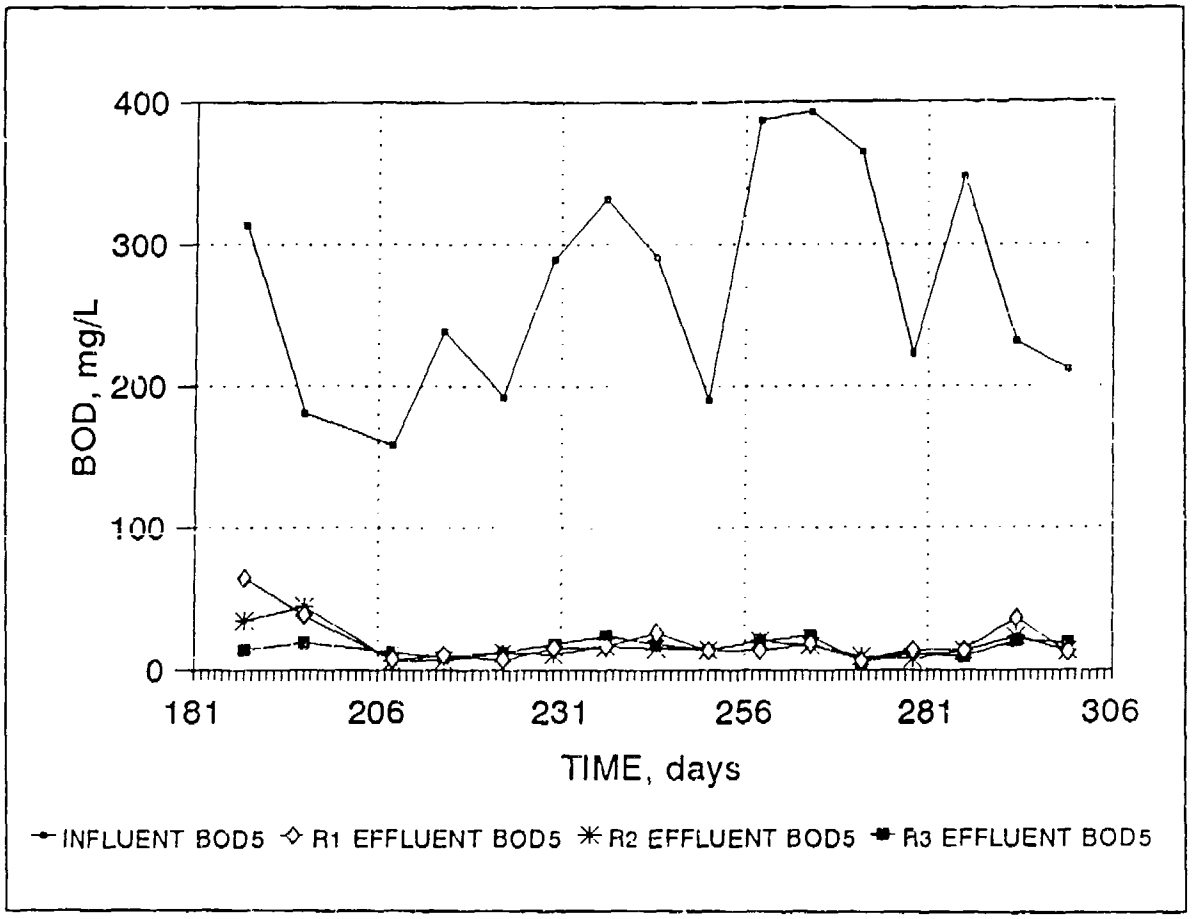



Figure 14. Hydrogen Peroxide Introduction Phase BOD data

Figures 16 and 17 present the MLSS and MLVSS data, respectively, for all three bioreactors while Bioreactor 3 was being introduced to residual hydrogen peroxide levels within the aeration chamber. These data support the results of the gross pollutant data in that the dosing of hydrogen peroxide did not appear to adversely impact bioreactor performance. Figure 18 presents the DO levels measured in Bioreactor 3 throughout the hydrogen peroxide Introduction Phase. Because air sparged into Bioreactor 3 continued throughout this study phase, the DO in the aeration chamber remained above 4 mg/ℓ until the air was completely turned off on Day 302. At this point, the DO within the Bioreactor 3 aeration chamber decreased to immeasurable levels.

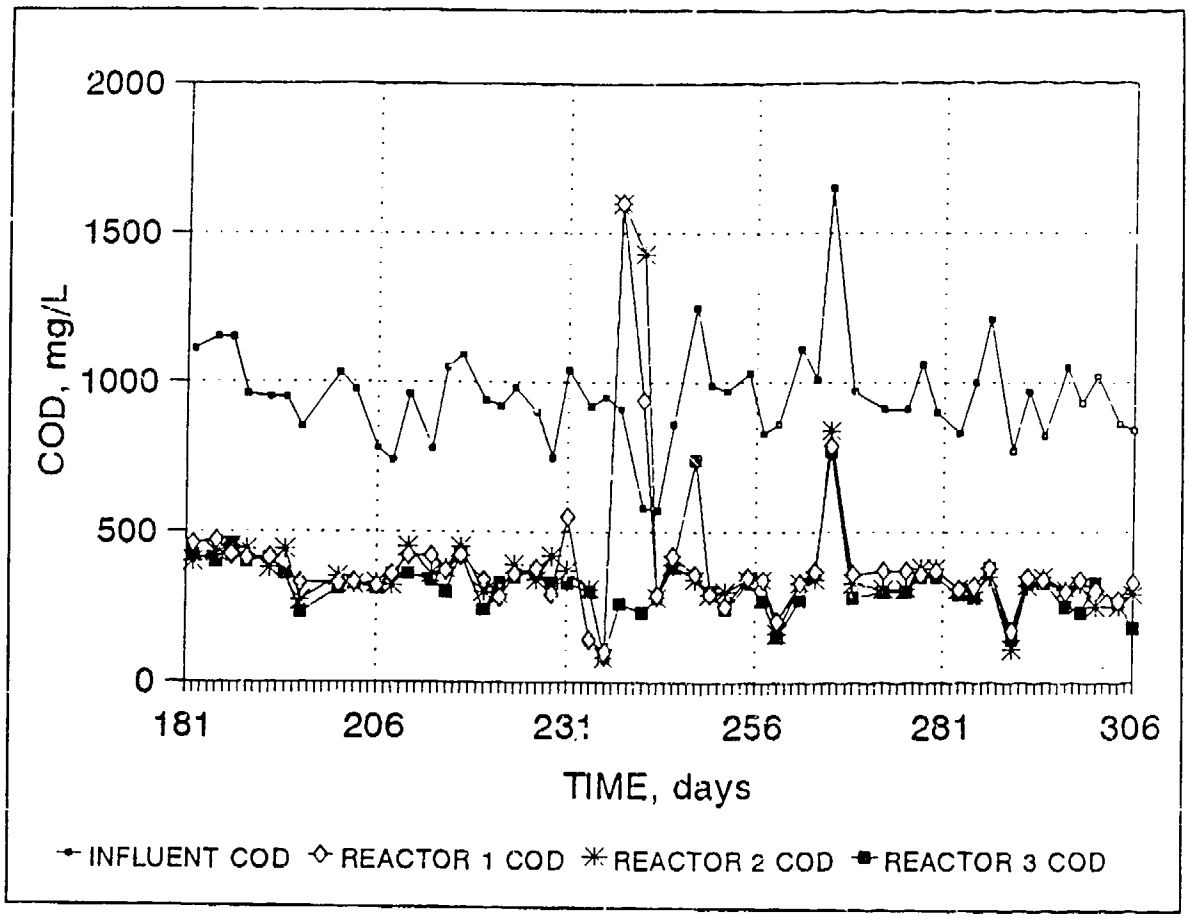



Figure 15. Hydrogen Peroxide Introduction Phase COD data

The air was not completely turned off until Day 302 because each time air sparging in Bioreactor 3 was ceased, the DO in the aeration chamber dropped below 0.5 mg/ℓ within 30 min even though hydrogen peroxide was being dosed into the bioreactor. Since standard engineering approximations suggest that the DO in the aeration chamber should always be maintained above 0.5 mg/ℓ, much concern was felt that the aerobic consortia will die off because of low DO levels. Also, during these experiments, residual hydrogen peroxide was not detected within the aeration chamber of Bioreactor 3. However, it was believed that the DO levels within the bioreactor were not measured using the DO meter nor were the residual hydrogen peroxide levels detected in the aeration chamber using the test strips because the dosing rate was applied at levels just high enough to satisfy the OUR of the bioreactor.

## Comparison of Bioreactor Performance

Bioreactor 2 was converted to pure oxygen sparging on Test Day 329. Therefore, from Day 329 to Day 523, the comparative performance of all three bioreactors were evaluated to determine if the use of hydrogen peroxide as an alternative oxygen source had any adverse effects on bioreactor performance.

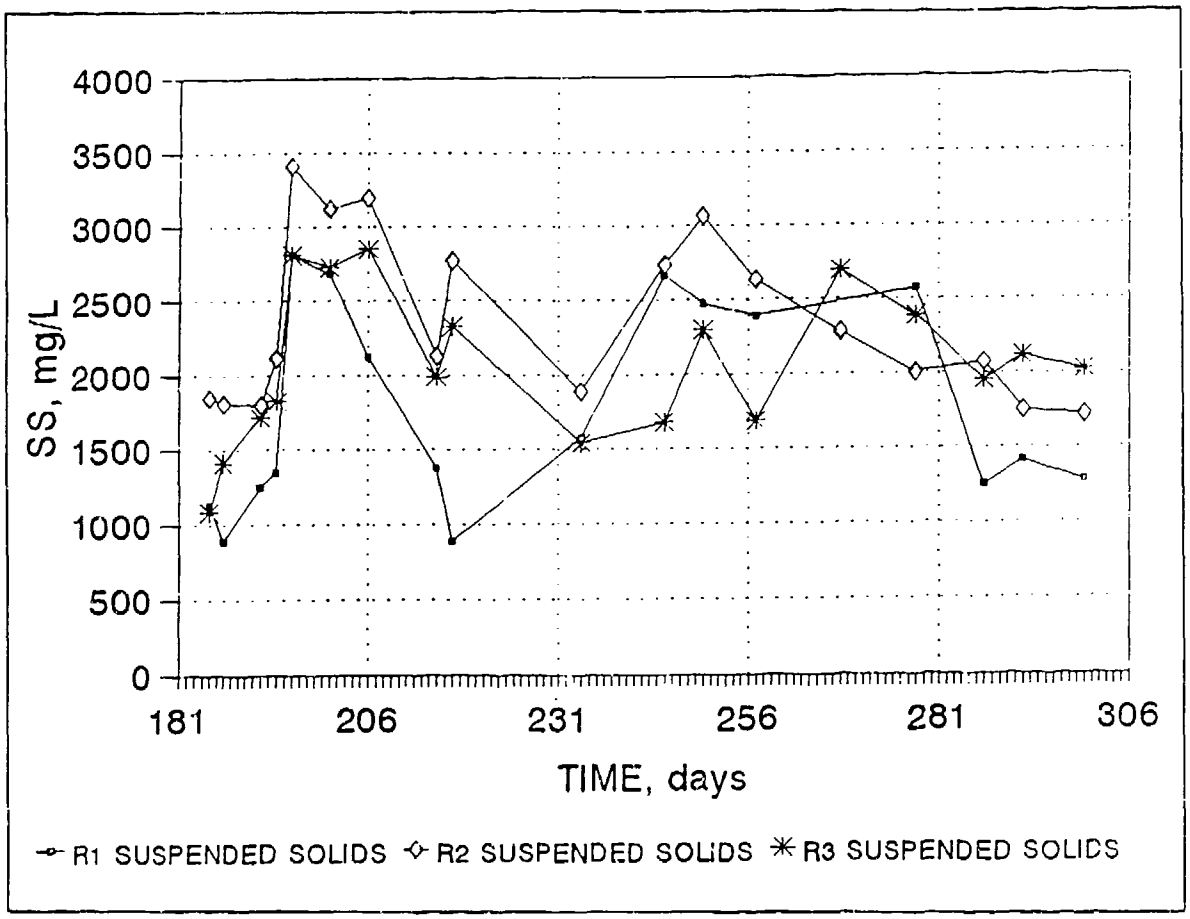



Figure 16. Hydrogen Peroxide Introduction Phase MLSS data

## SVI

Figure 19 presents the SVI data for the three bioreactors during the Comparison Phase. These data indicate that all of the bioreactors had sludge with poor settling characteristics. The high levels of benzene probably had an adverse effect on ML quality. The air sparged bioreactor (No. 1) generally had very high SVIs, usually greater than 1,000. Toward the end of the study phase, the settling properties of the Bioreactor 1 began to behave like the sludge from the other two bioreactors. The other two bioreactors (2 and 3) had SVIs that were much more stable than Bioreactor 1. These SVIs generally remained around the 450 to 500 range. Bioreactor 3 had elevated SVIs during the period from Day 380 through 400.

## MLSS

Figure 20 presents the MLSS data for the three bioreactors during the Comparison Phase. The MLSS concentrations measured varied dramatically by bioreactor during the first half of the comparison phase of the study. The

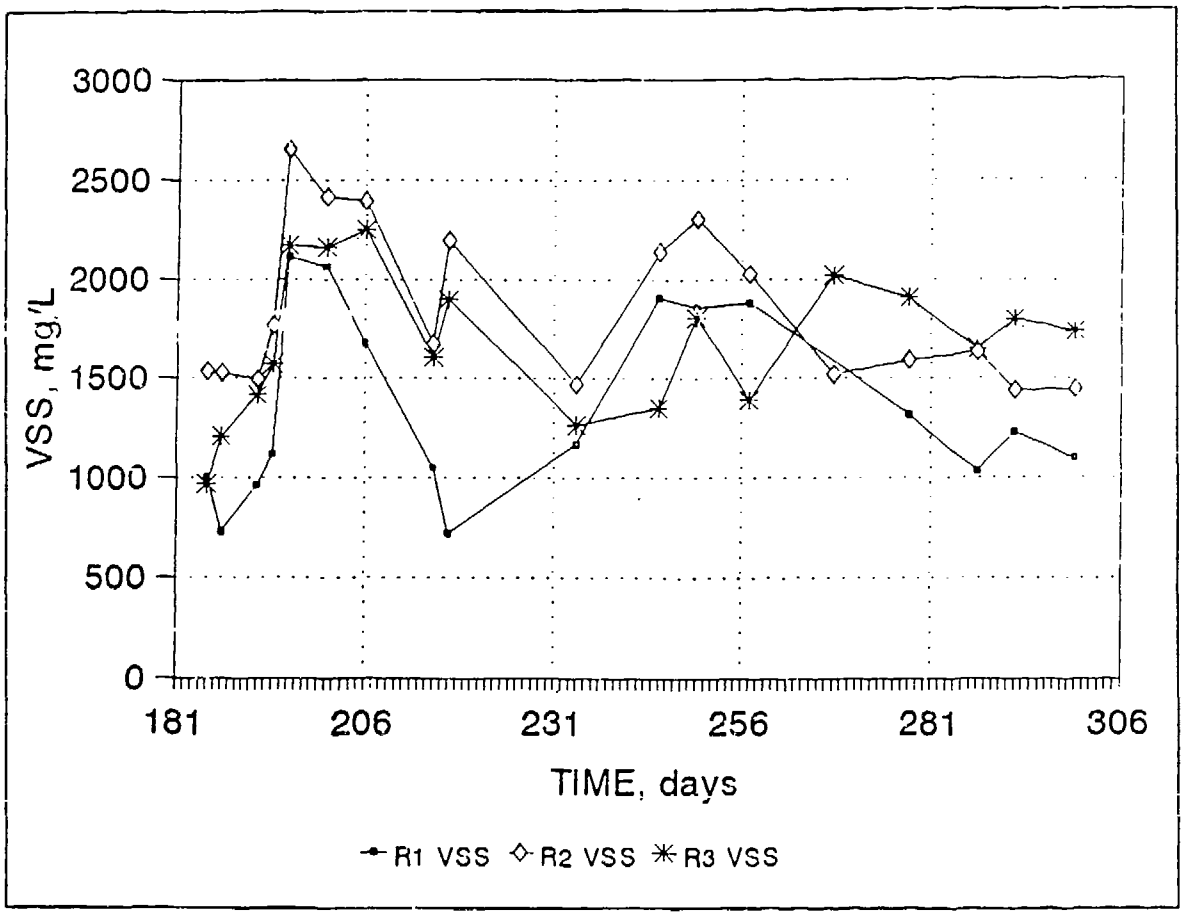



Figure 17. Hydrogen Peroxide Introduction Phase MLVSS data

MLSS concentration ranges for the air sparged, oxygen sparged, and hydrogen peroxide dosed bioreactors were approximately 500 to 1,500 mg/l, 2,750 to 1,500 mg/ℓ, and 800 to 2,200 mg/ℓ, respectively. However, by the second half of the study phase, the three bioreactors had similar MLSS levels (approximately 1,750 mg/ℓ). The reason for the variability in MLSS levels observed during the initial part of this phase is believed to be due to the poor settling characteristics of the ML. The SVI data supports this reasoning (see Figure 19). The air sparged bioreactor consistently had lower MLSS levels and higher SVIs than the other two bioreactors, with the oxygen sparged unit consistently having the highest MLSS and the lowest SVIs. Twice, the hydrogen peroxide dosed bioreactor MLSS concentrations declined then recovered. This decline appeared at the same time frame when the SVIs became elevated. Other than the two downward trends, the hydrogen peroxide dosed bioreactor (No. 3) had similar levels to the oxygen sparged bioreactor (No. 2).

## MLVSS

Figure 21 presents the MLVSS data for the three bioreactors during the Comparison Phase. These data generally followed the trends observed with

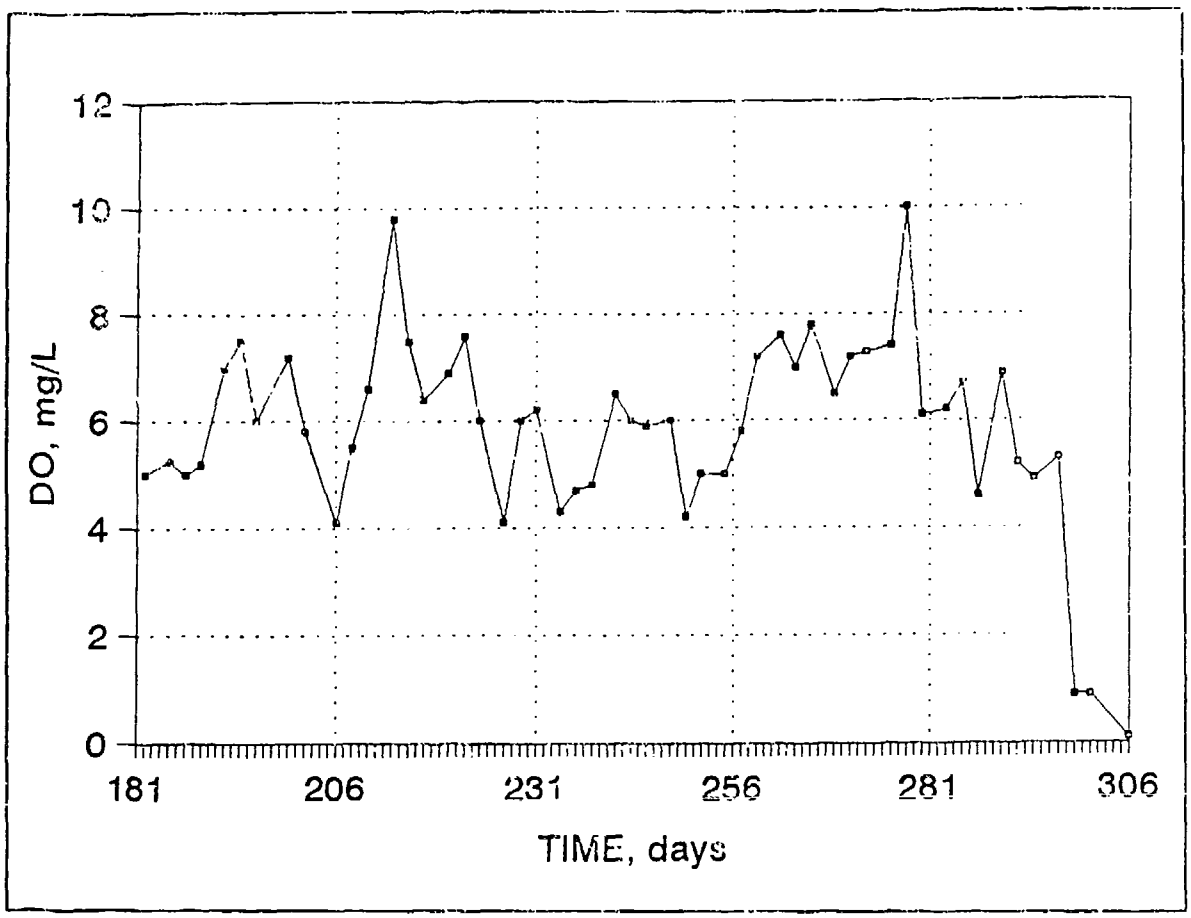



Figure 18. Hydrogen Peroxide Introduction Phase DO data

the MLSS data. The only appreciable difference noted between the MLSS and MLVSS data was toward the end of the phase when MLVSS levels in the air sparged bioreactor (No. 1) exceeded those of the hydrogen peroxide dosed bioreactor (No. 3).

## BOD

Figure 22 presents the BOD data for the three bioreactors during the Comparison Phase. These data indicate that BOD removal achieved from the three bioreactors was approximately equal. Percent removals of BOD over the course of this study phase were usually in excess of 95 percent, indicating good biological activity within all three bioreactors toward the influent BOD.

## COD

Figure 23 presents the COD data for the three bioreactors during the Comparison Phase. There was little difference in COD removal noted between the three bioreactors. This is somewhat surprising in light of the poor sludge

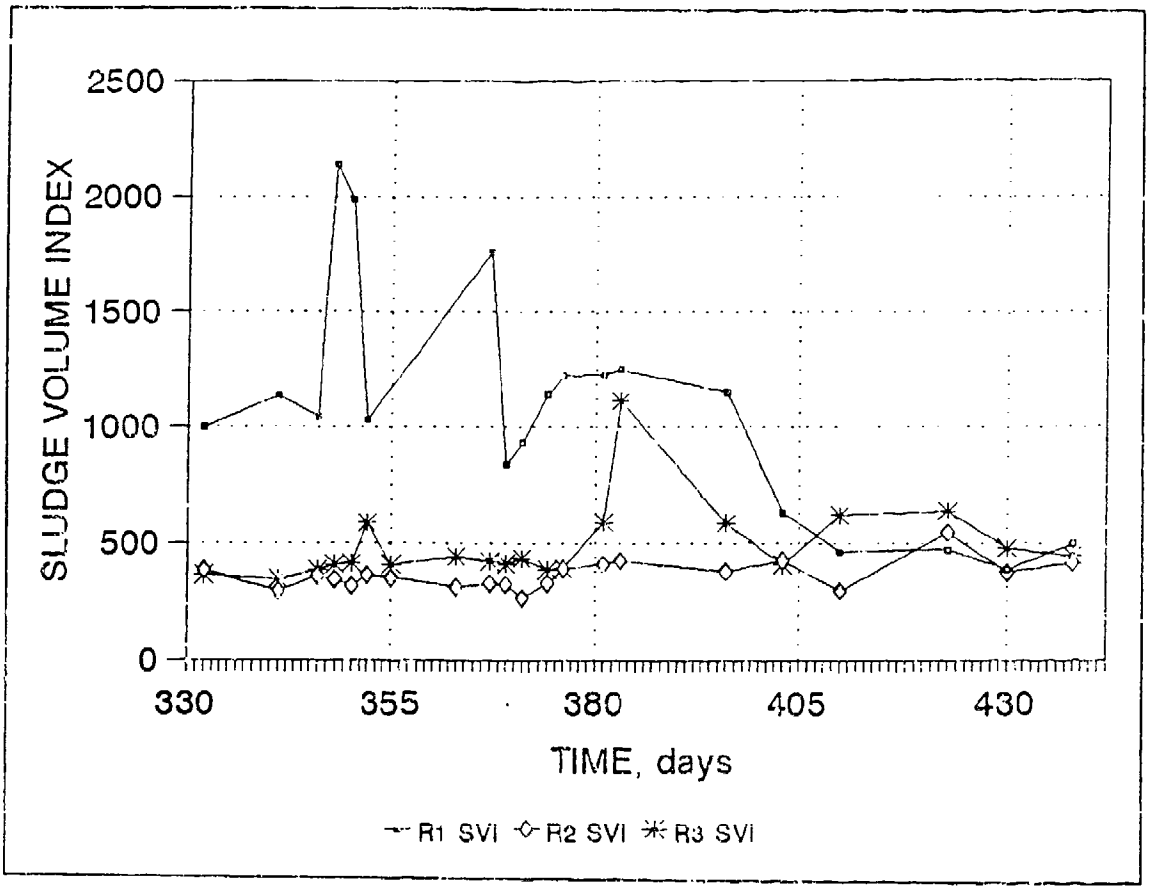



Figure 19. Comparison Phase SVI data

density and settling characteristics observed in Bioreactor 1 compared with the other two bioreactors.

## Benzene

As discussed earlier, Figure 2 presents the influent benzene concentrations analyzed over the course of this study including this study phase. As was the case with the Acclimation Phase, benzene was not detected in any of the bioreactor effluents.

## Off-gassing flux

Based on system parameters for each bioreactor system, a mass balance of benzene around the bioreactors was performed. The mass balance analysis assumed that all of the benzene was either biologically degraded in the bioreactor or stripped from the ML via the sparged gases (i.e., air or pure oxygen). Since a gas stream was not applied to Bioreactor 3, the hydrogen peroxide dosed bioreactor, the total gas flux exiting the bioreactor was assumed to be

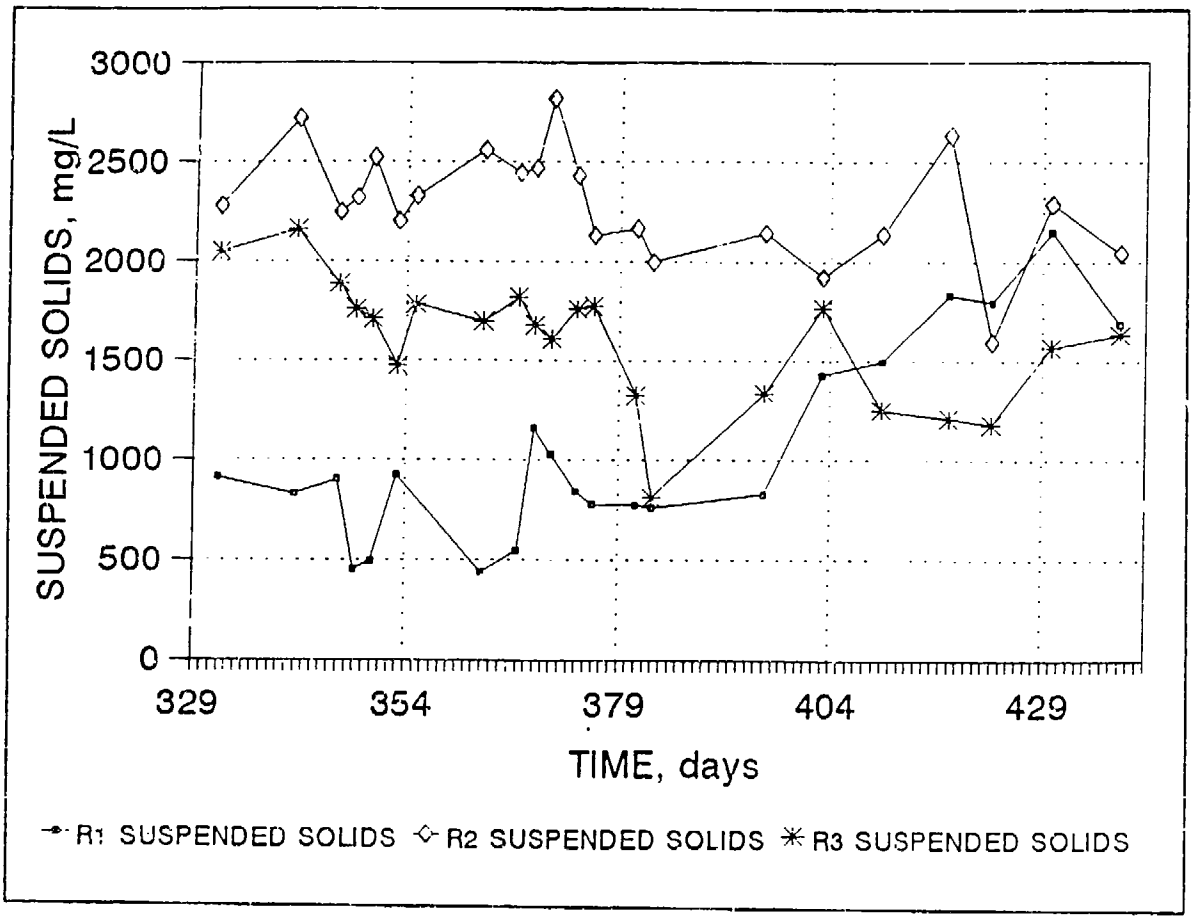



Figure 20. Comparison Phase MLSS data

zero. Benzene mass balance analysis estimated that the amount of benzene stripped from the air and oxygen sparged bioreactors (1 and 2) was approximately 15 and 1 percent, respectively. Since Bioreactor 3 had no gas streams exiting it, then the amount of benzene lost via stripping was zero.

## Nutrients

Figure 24 presents the ammonia concentrations for the influent and effluents for all three bioreactors as measured during the Comparison Phase These data indicate that the bioreactors generally had appropriate levels of ammonia for sustaining optimum biological activity. An interesting observation made upon further review of these data indicates that the hydrogen peroxide dosed bioreactor (Bioreactor 3) usually had much higher levels of ammonia present in its effluent. This trend indicates that Bioreactor 3 either had oxygen levels that were only capable of supporting microbial consortia degrading the carbon source and insufficient for the nitrifiers. Also, the residual hydrogen peroxide levels within the bioreactor may have had a detrimental effect on nitrifier populations, indicating that they are potentially more sensitive to the presence

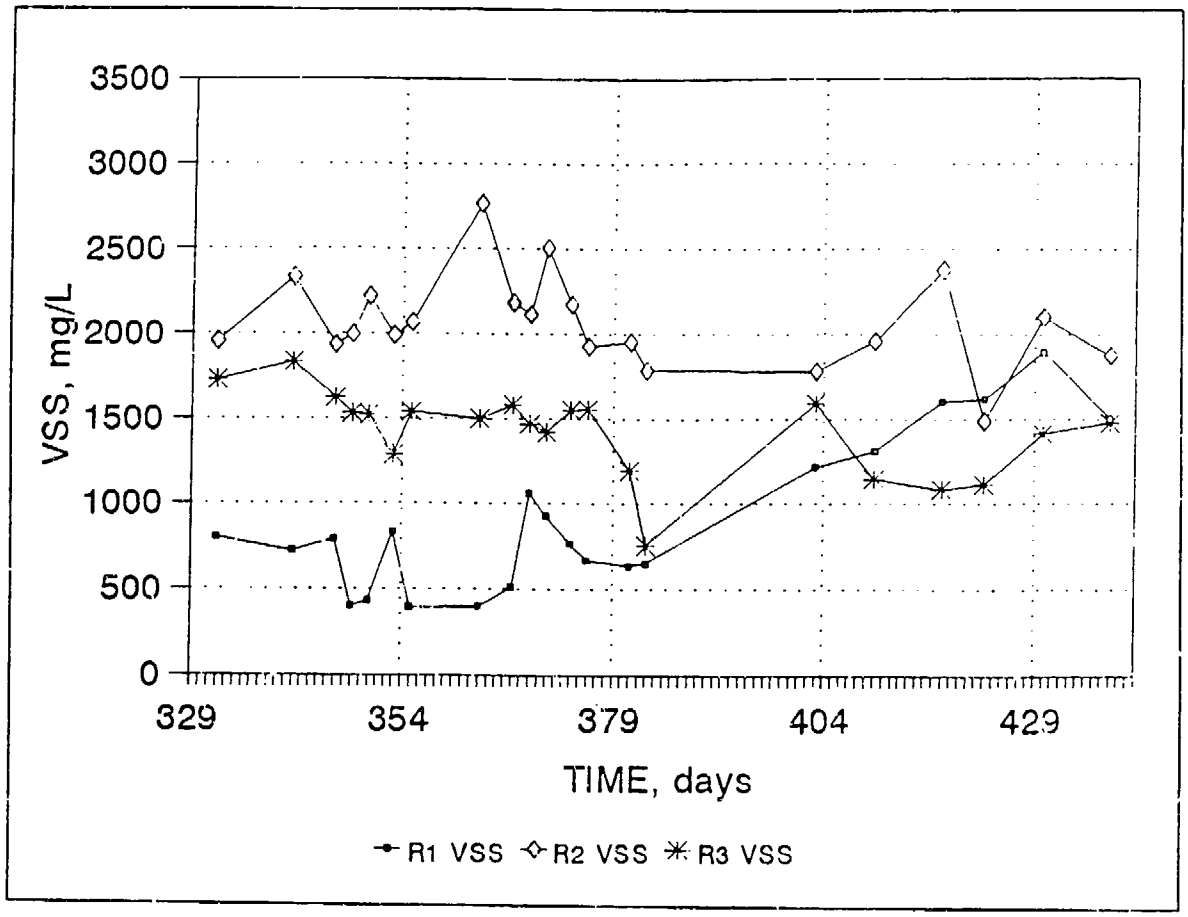



Figure 21. Comparison Phase MLVSS data

of chemical oxidizers. As a point of note, the phosphate nutrient data was not plotted because no significant trends were observed between phosphate levels. Phosphate in all three effluents remained at approximately 10 mg/ℓ throughout the experiments.

## Dissolved oxygen

Figure 25 presents the dissolved oxygen (DO) concentrations measured in the aeration chambers of the two gas sparged bioreactors during the Comparison Phase. These data indicate consistently high DO concentrations (approximately 7.5 mg/ℓ) were measured within the air sparged bioreactor (Bioreactor 1). The data also indicate that the DO levels maintained in the oxygen sparged bioreactor generally varied from 10 to 30 mg/ℓ. This high degree of variability was attributed to poor oxygen gas flow rate control obtainable using the regulatory valuing system supplied with the rotameter used. One interesting observation made during this phase of study was the lack of measurable DO within Bioreactor 3. This indicates that the hydrogen peroxide dose rate selected was generally at equilibrium with the OUR.

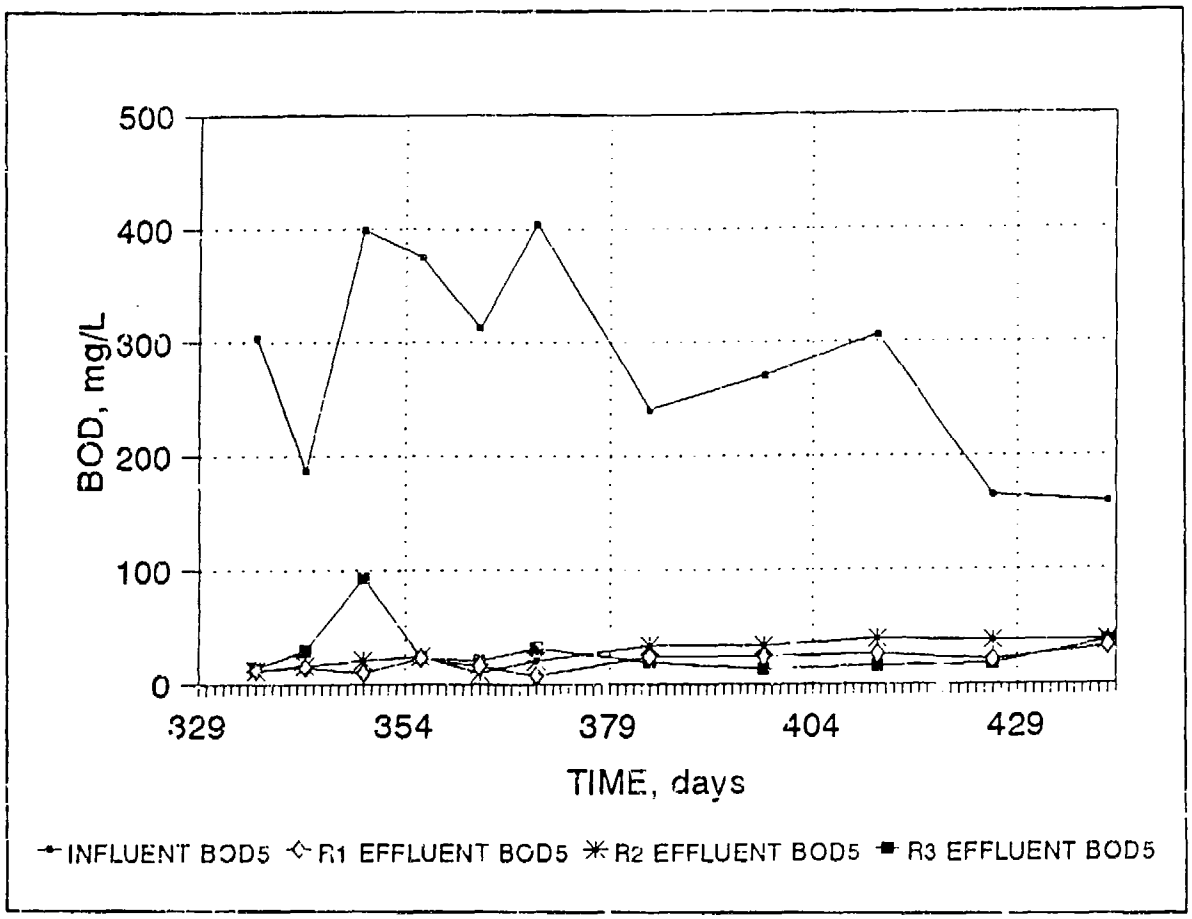



Figure 22. Comparison Phase BOD data

## Enzyme assays

The catalase assays were performed on filtered ML samples collected from Bioreactor 3. This water was analyzed for enzyme activity on the assumption that excessive amounts of enzyme activity will be observed in the Bioreactor 3 water as opposed to water collected from one of the other bioreactors. However, when little or no enzymatic activity was measured in Bioreactor 3 water, this assumption was considered invalid. The results of these experiments are summarized below.

Numerous repetitions of the catalase assay were performed using the filtered ML from Bioreactor 3. When the undiluted Bioreactor 3 filtered ML was used, the solution in the cuvette became cloudy, thus resulting in increased absorbance, rather than decreased as expected. The absorbance of the solution continued to increase over time, as very small crystals in the solution flocculated. After several hours, the precipitate settled, resulting in a clear liquid remaining in the upper portion of the cuvette. The resulting absorbance data is presented in Figure 26.

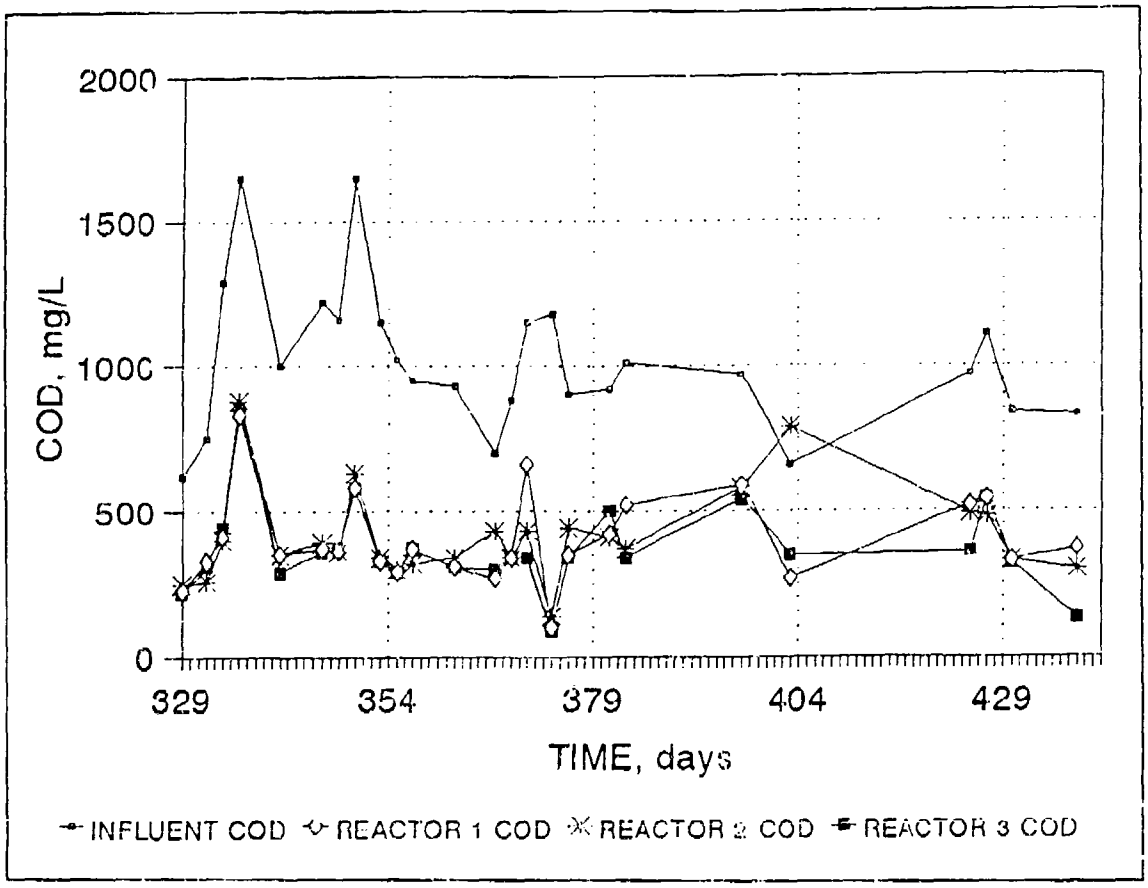



Figure 23. Comparison Phase COD data

When the Bioreactor 3 filtered ML was diluted by factors of one-half or one-tenth of the original concentration, no change in the absorbance was noted, indicating that no reaction took place. The resulting plots of absorbance versus time for the two diluted, filtered samples are presented as Figures 27 and 28, respectively. Both Figures 27 and 28 were compared with Figure 26 (the undiluted assay) with little or no catalase activity noted.

Based on the results of the assay, there appears to be little or no catalase or peroxidase activity associated with the filtered water taken from Bioreactor 3. The presence of either excessive amounts of catalase or peroxidase in the water phase of the ML would have resulted in the rapid degradation of hydrogen peroxide to water, with a resulting decrease in the absorbance of the sample.

The precipitate formed during several of the tests may be due to the reaction between the residual hydrogen peroxide and the organic compounds present in the reactor water. It is believed that the precipitate is some sort of protein present in the system that becomes insoluble in the presence of hydrogen peroxide.

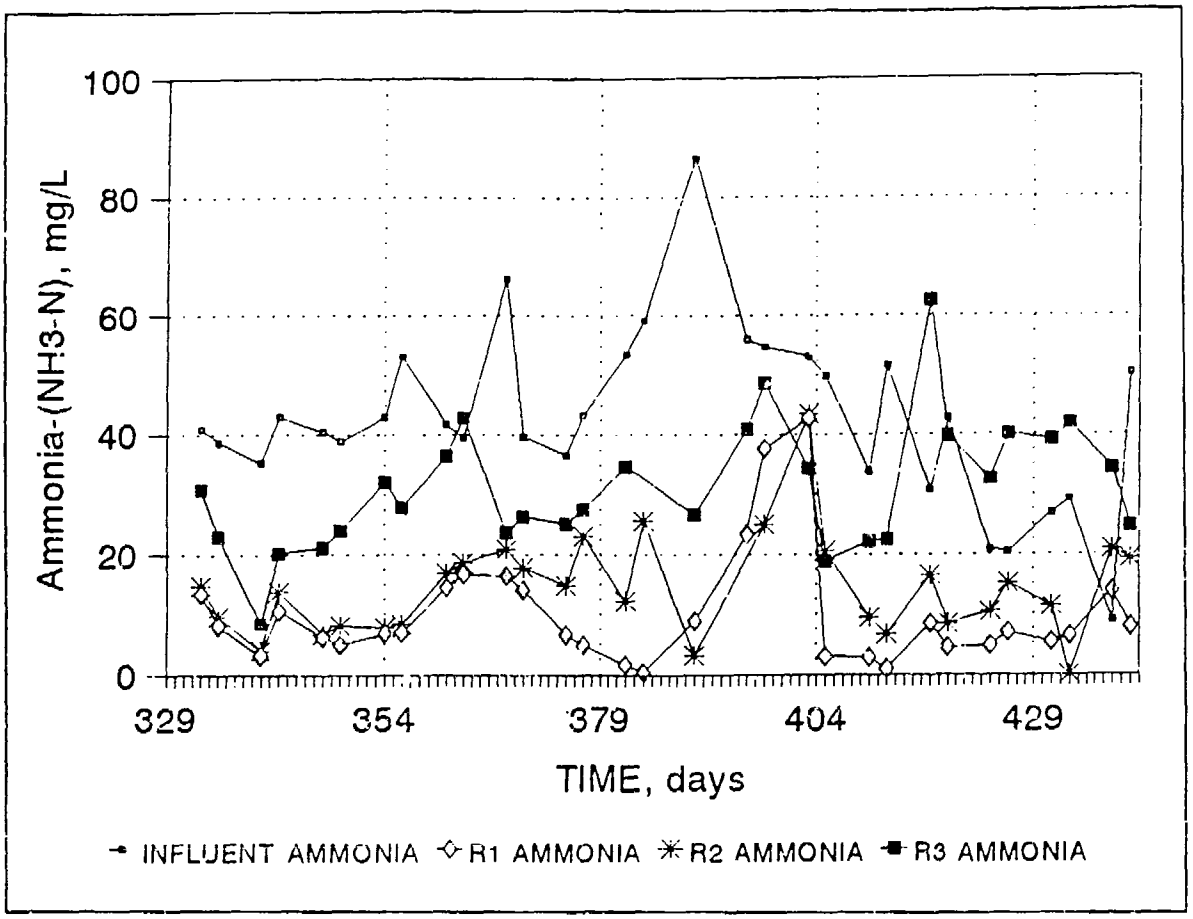



Figure 24. Comparison Phase ammonia data

From our assessment of enzymes responsible for oxidizer destruction, the destruction of hydrogen peroxide in Bioreactor 3 is primarily due to catalase or peroxidase production by the microorganisms in the ML. It is likely that the enzymes are produced by the microbes only when in direct contact with hydrogen peroxide; and since the water used in the assays was filtered, the microbial population was reduced to nearly zero, thereby removing all catalytic activity. Instead of attempting to analyze the enzymes in the unfiltered ML, which were expected to be extremely high, it was decided to use a different approach to quantify enzymatic degradation and subsequent utilization of hydrogen peroxide as an oxygen source. This approach involves quantification of enzymatic activity based on degradational kinetics obtained from batch testing, which is described in the subsequent section.

## Hydrogen peroxide fate

A variety of experiments were performed to determine the mechanisms of hydrogen peroxide degradation within Bioreactor 3. A variety of degradational pathways were evaluated through performance of a number of batch reaction experiments. Prior to investigating these pathways, a mass balance assessment

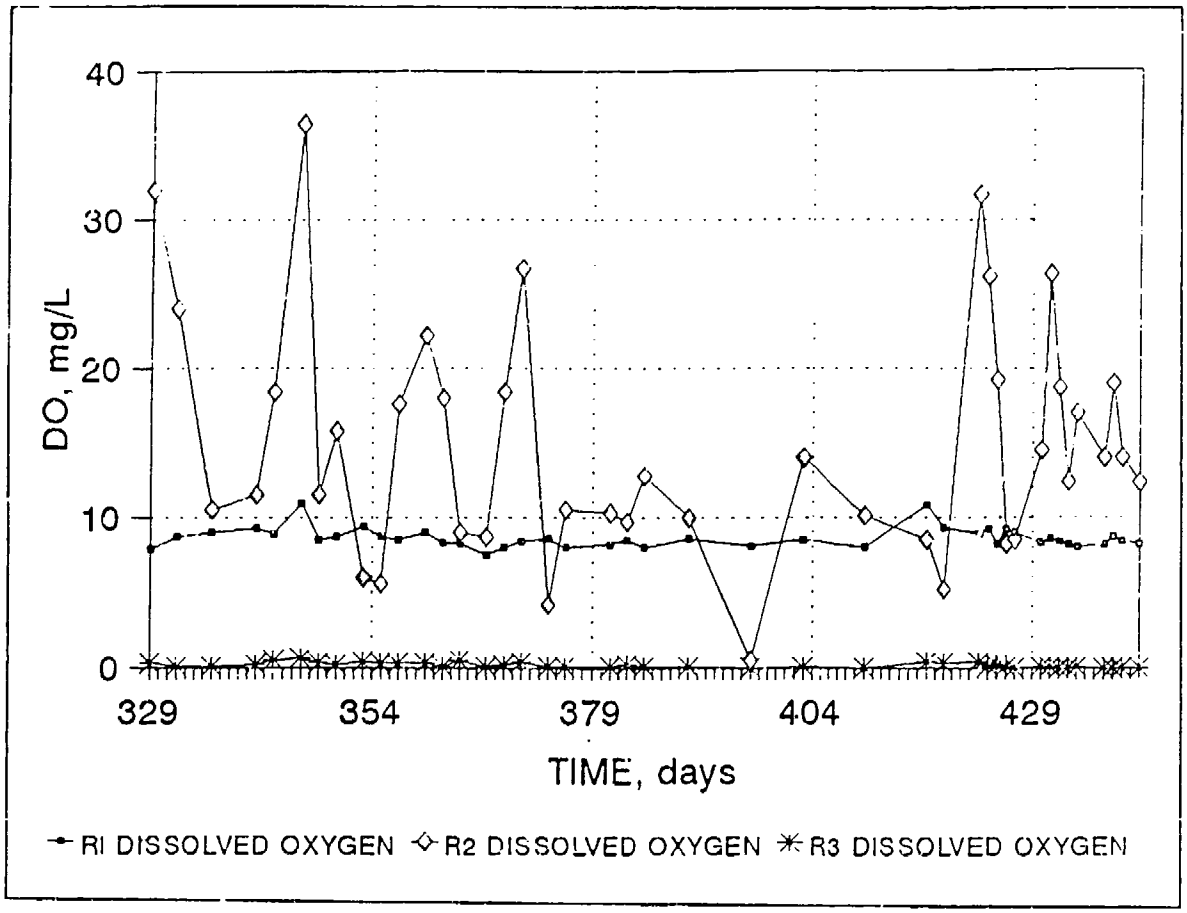



Figure 25. Comparison Phase DO data

of hydrogen peroxide was performed that included reaction terms (i.e., hydrogen peroxide was considered a reactive species). The mass balance equation for a system with hydrogen peroxide dosed into the bioreactor via hydrogen peroxide injection into the influent stream is presented below

$$\frac{dC_{EF}}{dt} = \frac{Q \times C_o}{V_r} - \frac{Q \times C_{EF}}{Vr} - K_1 \times C_{EF} \times \text{MLVSS} - K_2 \times C_{EF} \times C_s$$

where

$Q$ = system flow rate, l/min

$K_1$ = MLVSS/$H_2O_2$ reaction rate constant, $(\text{mg}/\ell \times \text{min})^{-1}$

$K_2$ = sink/$H_2O_2$ reaction rate constant, $(\text{mg}/\ell \times \text{min})^{-1}$

$C_s$ = summation of concentrations of abiotic hydrogen peroxide sinks, such as contaminant(s), amendments, iron, and carbonate species, mg/$\ell$

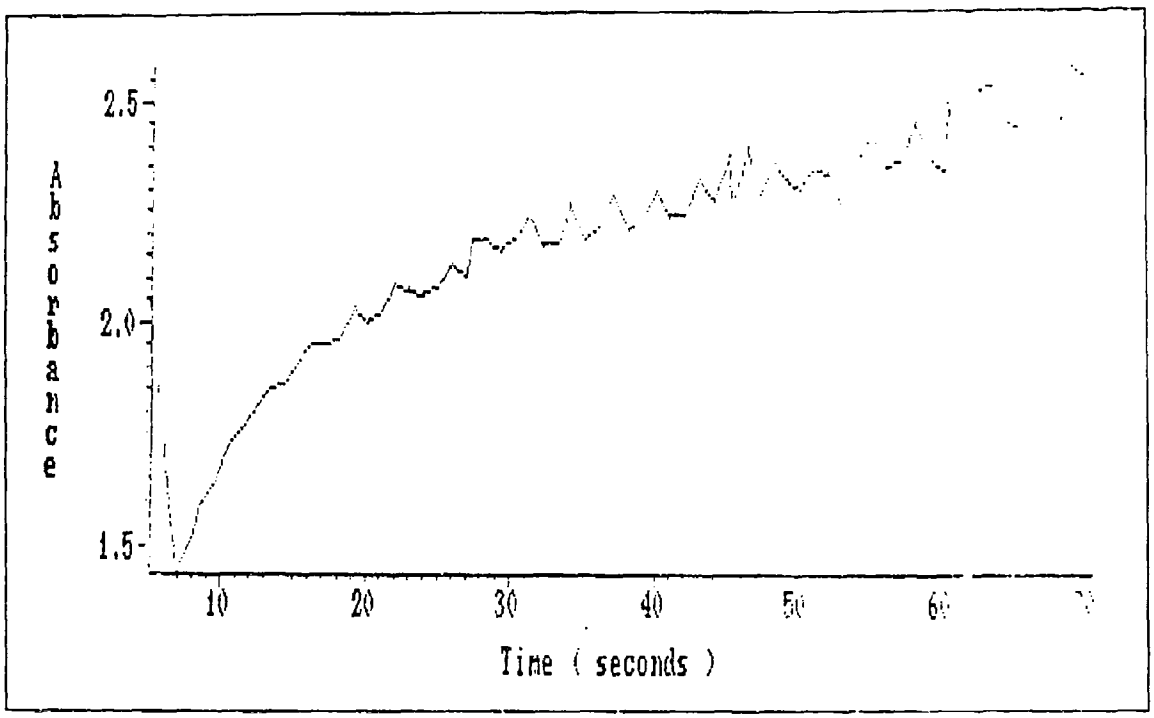



Figure 26. Spectrophotometric trace of unfiltered influent

To convert this equation into an equation that models systems injecting hydrogen peroxide directly into the aeration via a separate injection flow, simply replace the mass input terms, $Q$ and $C_o$, with the flow rate from the stock hydrogen peroxide tank, $Q_{HP}$, and the concentration of hydrogen peroxide in the stock tank, $C_{HP}$.

Note that the above degradation terms are based on an assumed overall second order reaction. This assumption is typical of oxidation reactions and will be explored further in subsequent discussions. Steady-state conditions and pseudo first order reaction kinetics for the sink term were assumed, thereby, resulting in the conversion of the reactive mass balance equation to

$$^{SS}C_{EI} = \frac{Q \times C_o}{Q + (K_1 \times V_r \times \text{MLVSS}) + (K_s \times V_r)}$$

where $k_s$ = pseudo first order rate constant, $\text{min}^{-1}$, which is theoretically equal to

$$k_s = K_2 \times C_s$$

For the reactive mass balance steady-state model to be of value to the design community, the rate constants, $K_1$ and $k_s$, must be determined. The

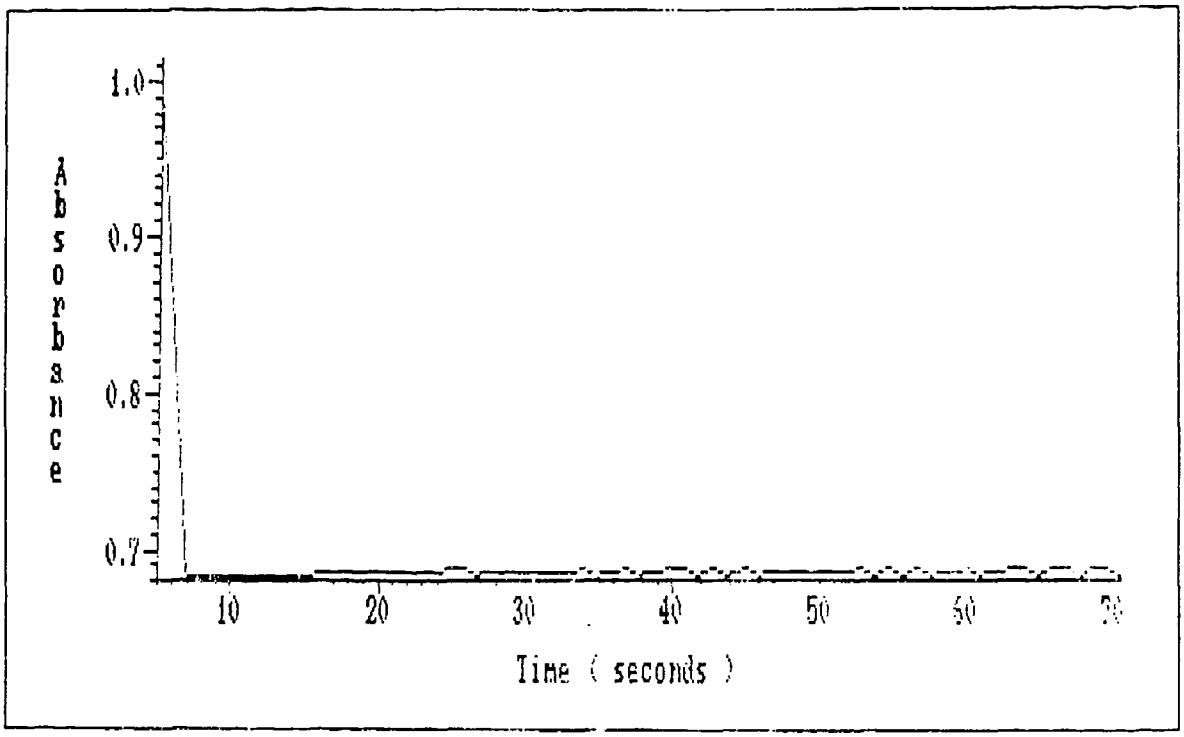



Figure 27. Spectrophotometric trace of filtered, one-half diluted influent

following discussions present the results of the experiments to determine the numerical value of these constants.

## Reaction with ML biomass (biotic degradation)

Batch hydrogen peroxide reaction experiments were performed using ML samples collected from the bioreactors from another ongoing WES study. The MLVSS used was 2,209, 1,105, 553, and 276 mg/ℓ. Hydrogen peroxide was dosed at 100 mg/ℓ. Results from these experiments were graphed on a semilog plot of dimensionless hydrogen peroxide concentration ($C/C_o$) versus test time in minutes (Figure 29). Each data set represents an average of two replicate runs. A curve fitting effort of the data was undertaken that indicated that a straight line was a good fit (all regressions had correlation of fit constants [$r^2$] greater than 0.90). A straight line fit indicates that the degradation kinetics appear to be first order with respect to hydrogen peroxide. By definition of first order kinetics, the slope of a linear plot using a semilog plot is the numerical value for the first order rate constant. Based on the units selected for these experiments, the units associated with the rate constants are inverse time ($min^{-1}$). The first hydrogen peroxide degradation rate constants for each of the MLVSS levels tested, 2,209, 1,105, 553, and 276 mg/ℓ, were 0.453, 0.218, 0.091, and 0.047 $min^{-1}$, respectively. These rate constants were then plotted on a standard cartesian plot against the various MLVSS values yielding

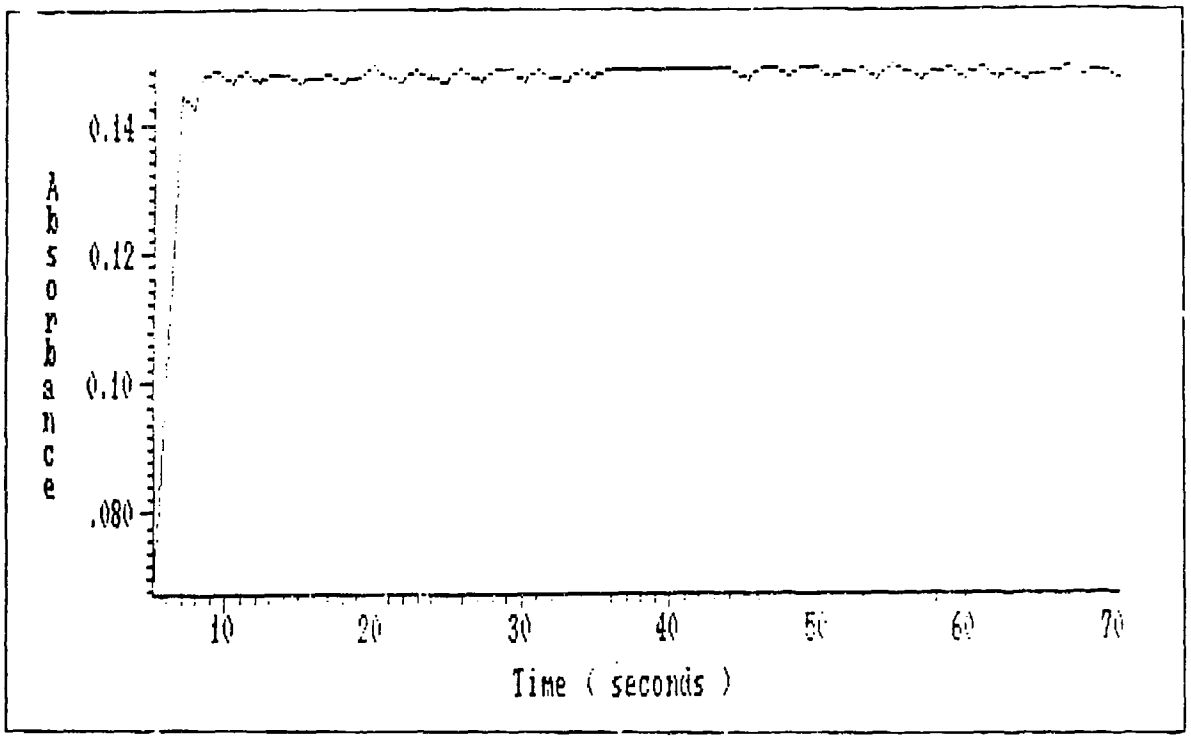



Figure 28. Spectrophotometric trace of filtered, one-tenth diluted influent

a straight line (Figure 30). The straight line plot of the rate constants versus MLVSS values indicates that the overall reaction order of hydrogen peroxide and MLVSS is second order, and first order with respect to both hydrogen peroxide and MLVSS. Since the first order rate constant is equal to a function of the overall reaction rate constant $K_l$ and MLVSS concentrations (i.e., $k = K \times \text{MLVSS}$), the slope of the line in Figure 30 is numerically equal to the overall reaction rate constant $K_l$, which was determined to be 0.00022 $(\text{mg}/\ell \times \text{min})^{-1}$ with a correlation of fit $r^2$ of 0.86.

## Reaction with groundwater constituents and influent amendments (abiotic reactions)

Additional batch experiments were performed to determine if other non-regulated chemicals, whether added as nutrients or already present as contamination or natural constituents, significantly impacted hydrogen peroxide fate by serving as sinks. Two water samples were used in these series of experiments. The first sample was distilled water that was dosed with the same amendments in respective amounts that were added to the groundwater influent (i.e., acetate, sugar, and N/P nutrients). This experiment evaluated the reaction of hydrogen peroxide with the influent amendments. The second experiment used actual RMA groundwater that was not amended with the additives. This experiment essentially evaluated the reaction of hydrogen peroxide with the benzene in the groundwater. Figure 31 presents the results of these experiments, which indicate that these reactions are relatively slow compared with the reaction of

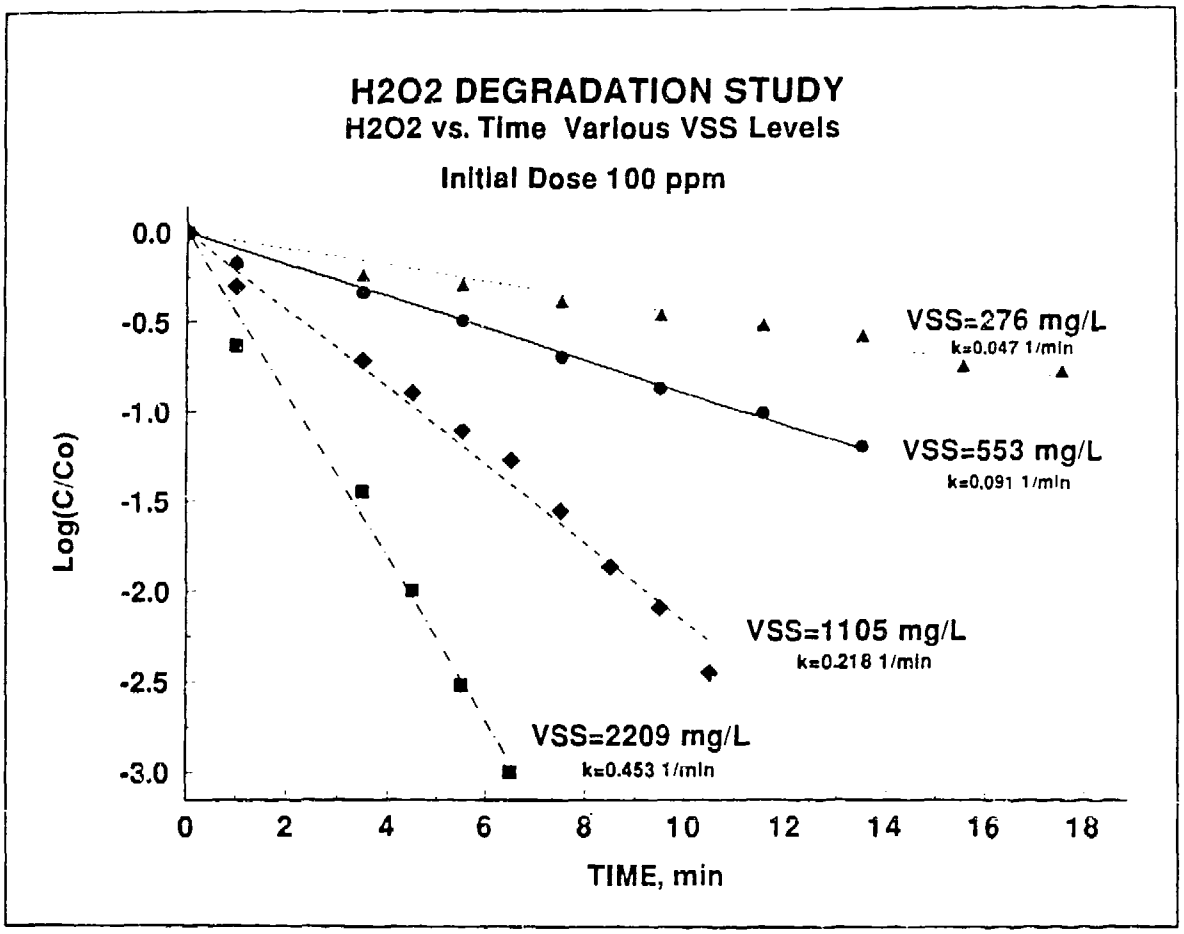



Figure 29. Biotic degradation of hydrogen peroxide with various MLVSSs versus reaction time

hydrogen peroxide with the ML biomass. The pseudo first order rate constants for the amendments and groundwater chemicals were 0.01336 and 0.00234 $min^{-1}$, respectively ($r^2$ for these linear regressions were both in excess of 0.75). The two rate constants were added together to equal the overall, pseudo first order rate constant $k_s$.

## Hydrogen peroxide fate model

The final form of the steady-state hydrogen peroxide concentration within the suspended growth bioreactor treating contaminated groundwater is presented below,

$$^{SS}C_{EI} = \frac{Q \times C_o}{Q + (0.0022 \times V_r \times \text{MLVSS}) + (0.0159 \times V_r)}$$

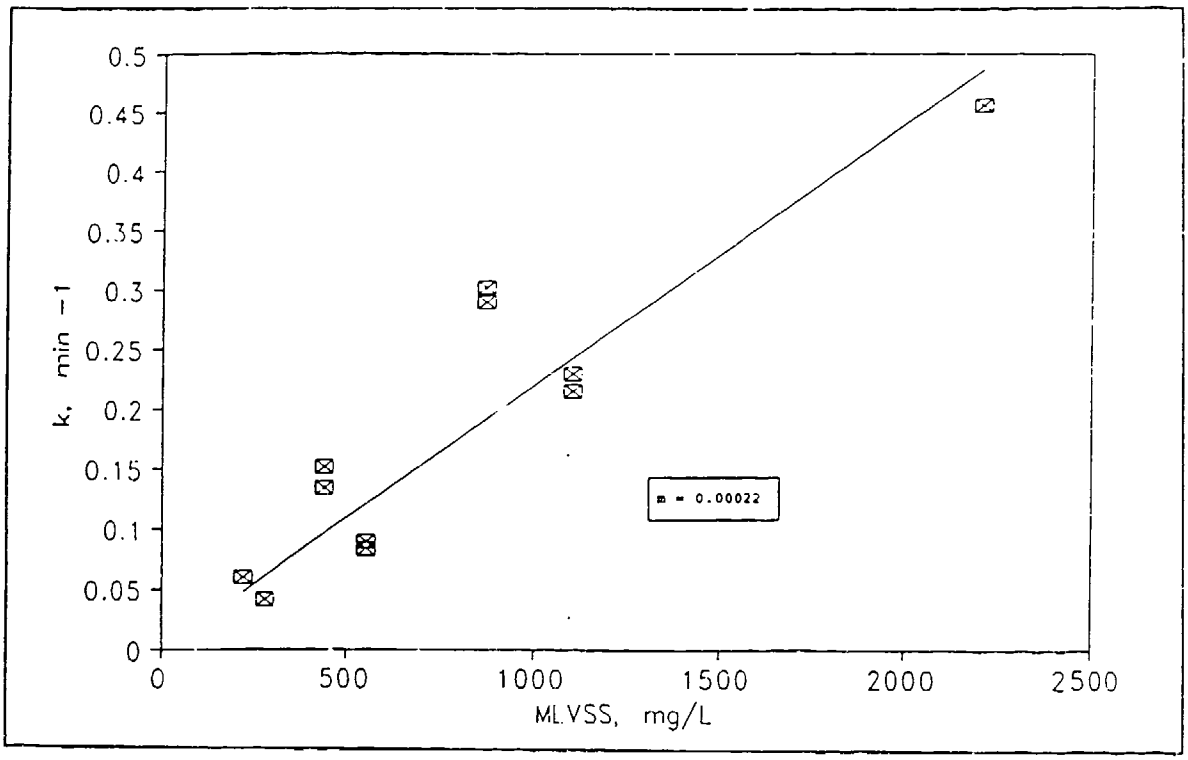



Figure 30. First order reaction rates for 100-mg/ℓ $H_2O_2$ versus MLVSS

The above model is valid as long as the residual hydrogen peroxide dose or point of hydrogen peroxide injection does not have a significant inhibitory or toxic effect on the ML biomass activity. To use this model for application to other groundwater systems, the sink rate constants specific to each water source must be experimentally determined. Further refinement to the model could be made by converting the sink term from a pseudo first order to a second order term that has dependence on general water chemistry. This will give the model a more universal application with little or no experimental requirements prior to its use. Unfortunately, this effort was beyond the scope of this study. However, ongoing research activities at WES will address this issue.

Other potential uses of this model for environme gineers are for estimating hydrogen peroxide fate in an aquifer undergoing in situ biotreatment by incorporating this model into an overall groundwater model, or estimating the required hydrogen peroxide dose into an AS system with low DOs as a result of an input of a highly contaminated slug of organic material. Hydrogen peroxide dosing is also commonly done by municipal sewage plants to raise aeration tank DO levels during the summer to reduce anaerobically produced odors.

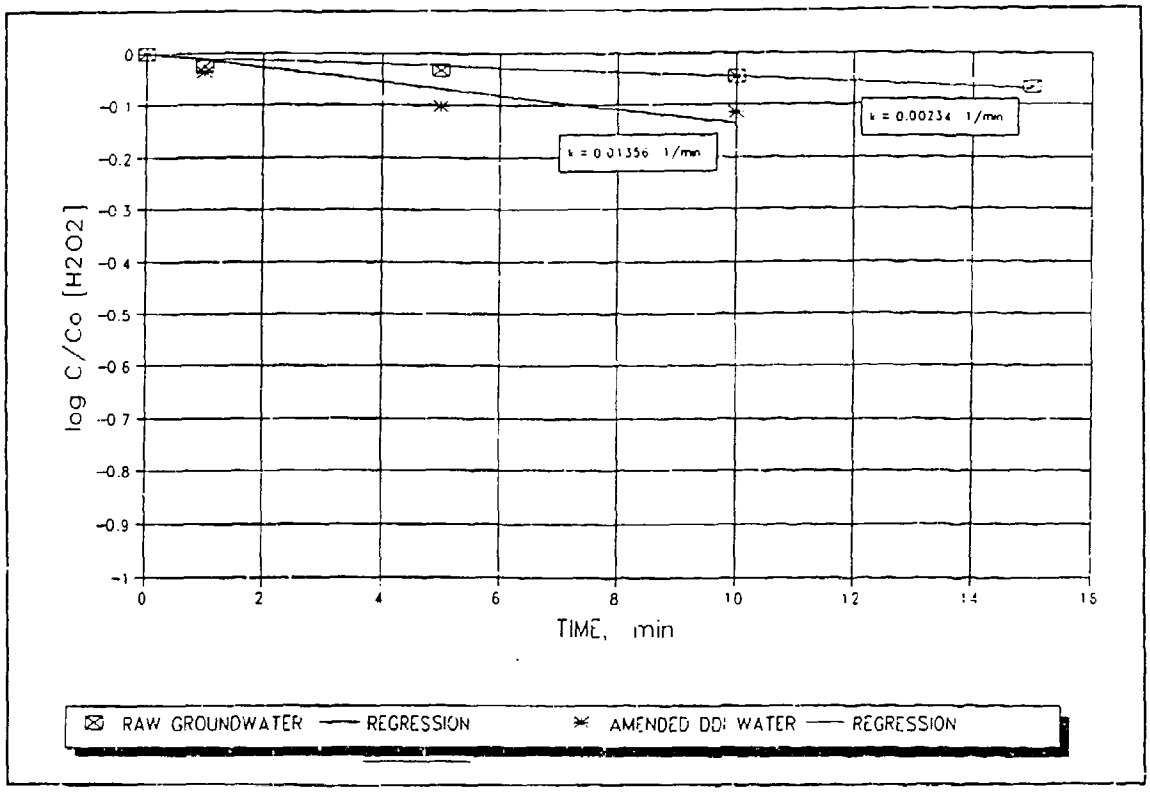



Figure 31. Abiotic degradation of 100-mg/ℓ $H_2O_2$ versus test time

### Summary

The elimination of a contaminated gas stream exiting the bioreactor with little or no sacrifice to bioreactor performance was the essence of this study. The results presented from this study phase prove the technical feasibility of using hydrogen peroxide as an alternative oxygen source for AS systems treating waters containing VOCs that may be stripped. Also, a hydrogen peroxide fate model was proposed and developed. The data used for developing the model indicate that the major fate term for hydrogen peroxide is biotic reactions.

## Optimization Phase

Based on these results, an Optimization Phase was initiated to determine the optimum residual hydrogen peroxide dose. Also, an economic evaluation of hydrogen peroxide dosing as an alternative oxygen source, as compared with both air and oxygen peroxide sparging, was performed. As previously stated, the dosing rate selected in the Comparison Phase was based on the measured OUR for the air sparged bioreactors. Three residual hydrogen peroxide levels were evaluated during the Optimization Phase: 100 mg/ℓ, 50 mg/ℓ, and 10 mg/ℓ. Prior to the dosing of the three bioreactors with the three candidate

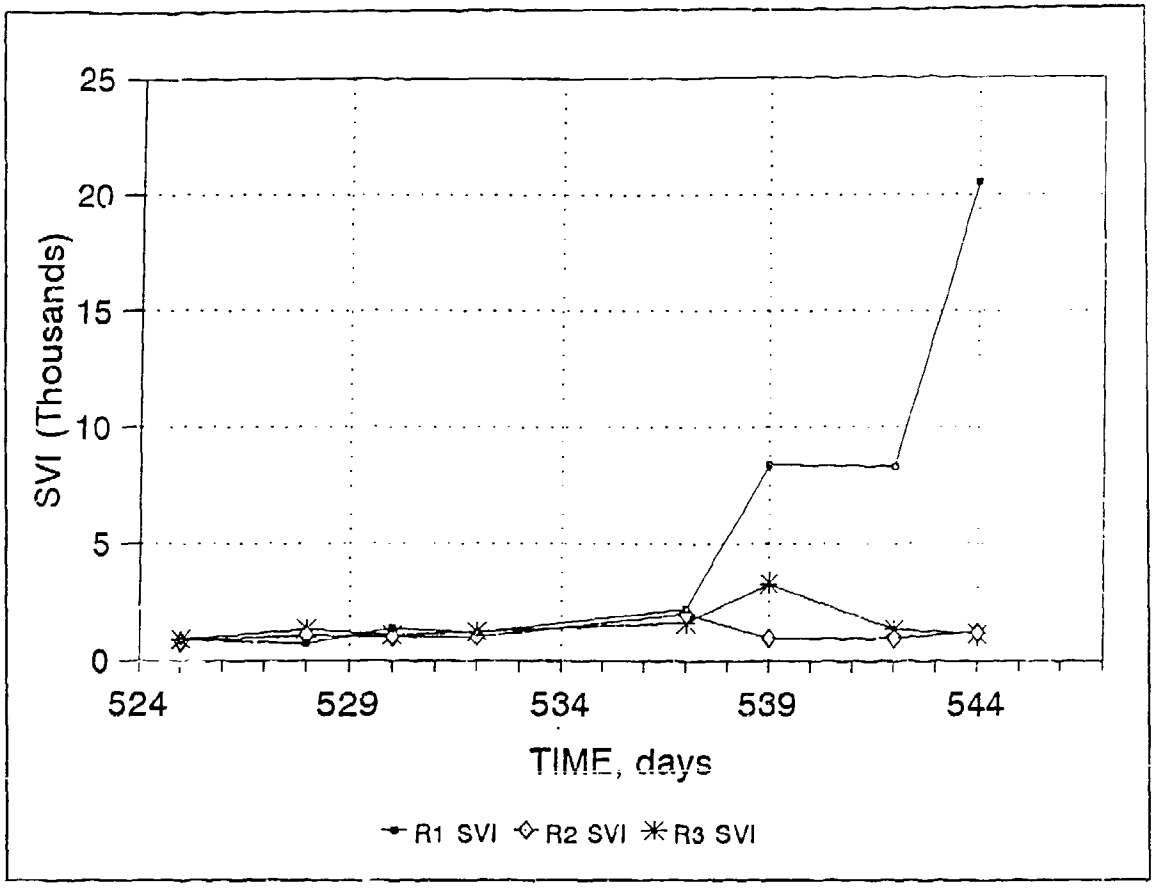



Figure 32. Optimization Phase SVI data

dosages, the contents from all three bioreactors were mixed together to ensure that each bioreactor had approximately the same biomass at the initiation of this study phase. The impact of each hydrogen peroxide dose rate on the performance of the bioreactors are discussed below.

## SVI

Figure 32 presents the SVI data for the three bioreactors during the Optimization Phase. These data indicate that within 2 weeks of operating Bioreactor 1 under an applied residual hydrogen peroxide dose of 10 mg/ℓ, the settling characteristics went from poor to almost nonsettling. During this same period, the measured SVI of the ML from the other two bioreactors (2 and 3) remained constant at approximately 500.

## MLSS

Figure 33 presents the MLSS data for the three bioreactors during the Optimization Phase. The MLSS data indicate that biomass in Bioreactors 2 and 3

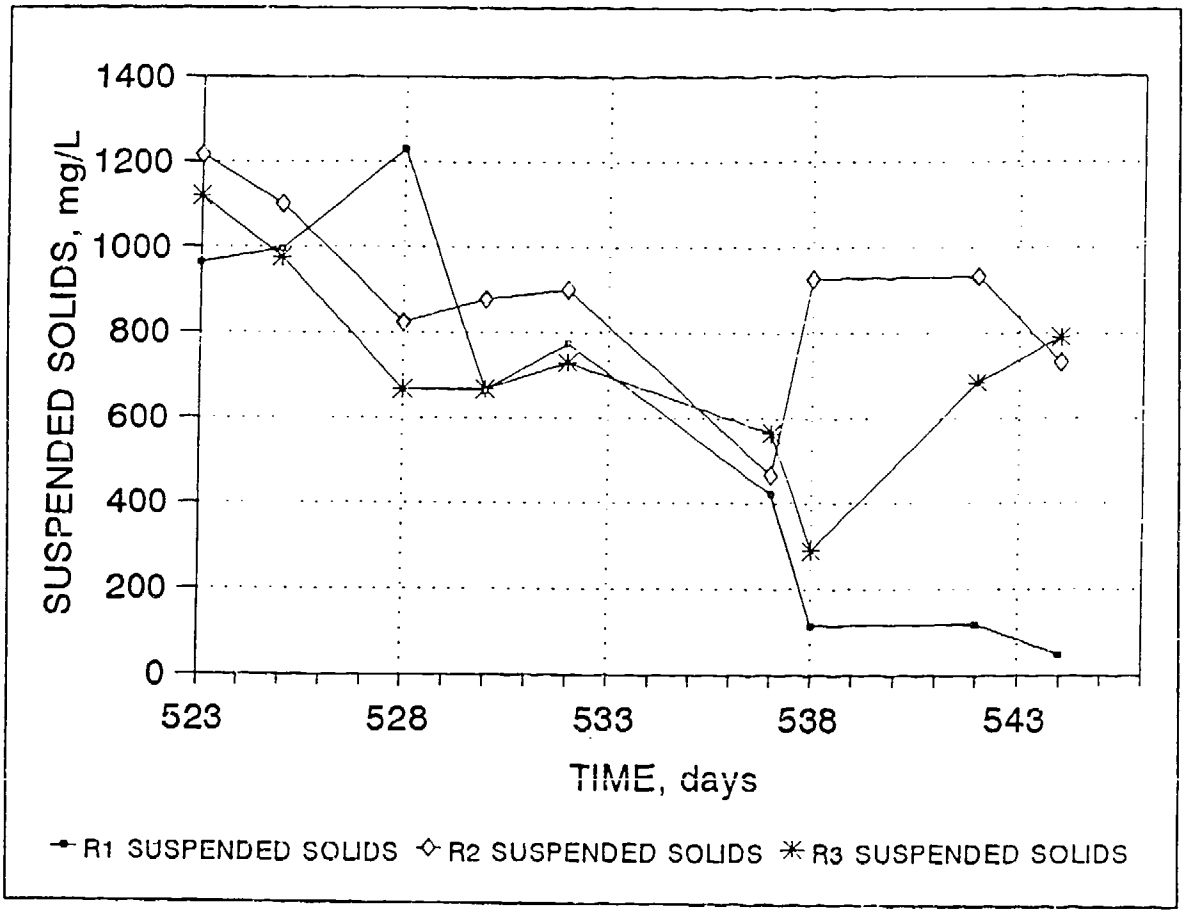



Figure 33. Optimization Phase MLSS data

remained relatively stable over the course of this study phase. On Day 537, an interruption in the influent feed because of malfunctioning of the influent pumps resulted in an upset in Bioreactors 2 and 3 and resulted in a slight solids loss. However, both bioreactors recovered with an increase in MLSS to levels prior to the upset in influent feed. On the other hand, Bioreactor 1 experienced continual MLSS reductions throughout the Optimization Phase. By Day 543, Bioreactor 1 was considered biologically dead because MLSS dropped to levels below 100 mg/ℓ.

## MLVSS

Figure 34 presents the MLVSS data for the three bioreactors during the Optimization Phase. These data followed the same trends observed with the MLSS.

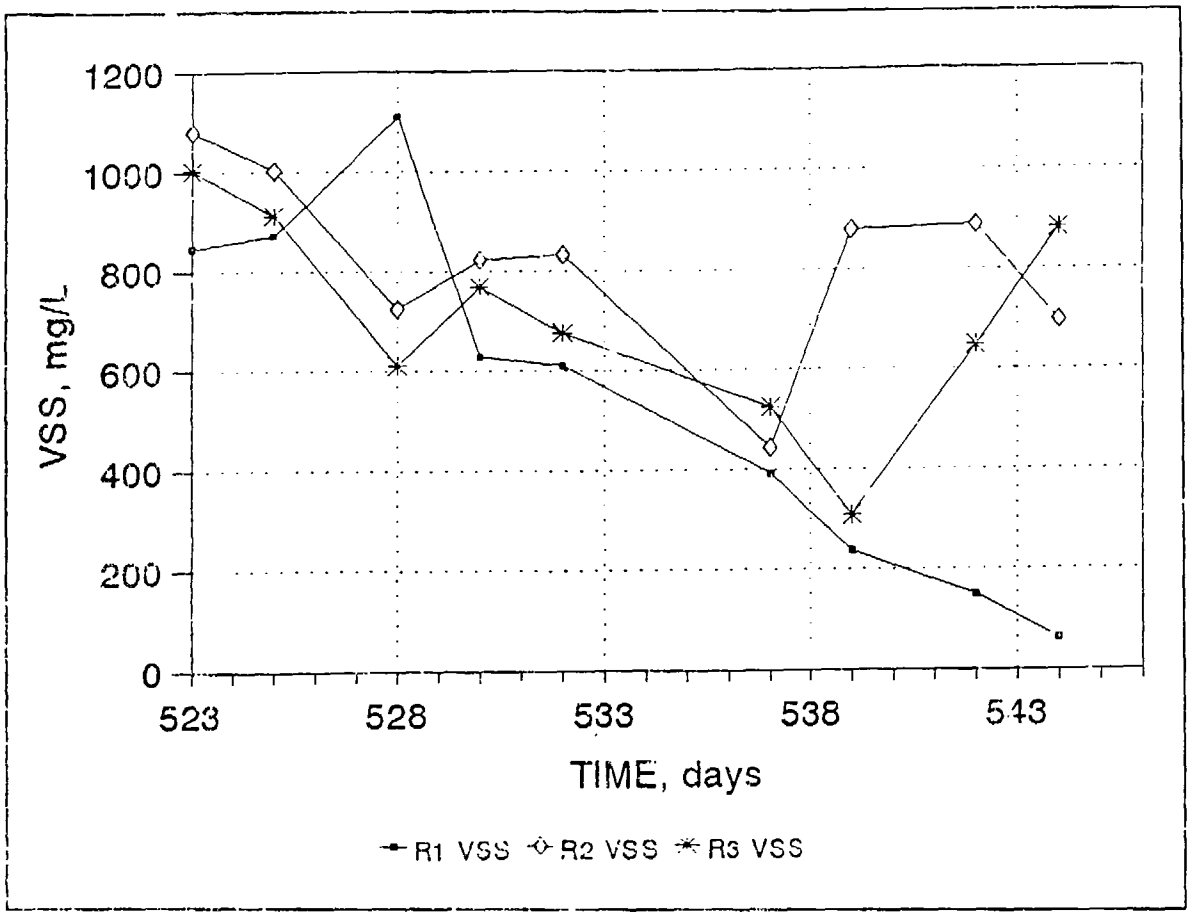



Figure 34. Optimization Phase MLVSS data

## COD

Figure 35 presents the COD data for the three bioreactors during the Optimization Phase. The COD data indicate that the rapidly decreasing solids concentrations did not appear to significantly impact COD removal. This trend is surprising, especially in light of the extremely low solids concentrations measured toward the end of the Optimization Phase (Day 543).

## Benzene

As was the case with the other test phases, benzene was not detected in any of the bioreactor effluents, including Bioreactor 1.

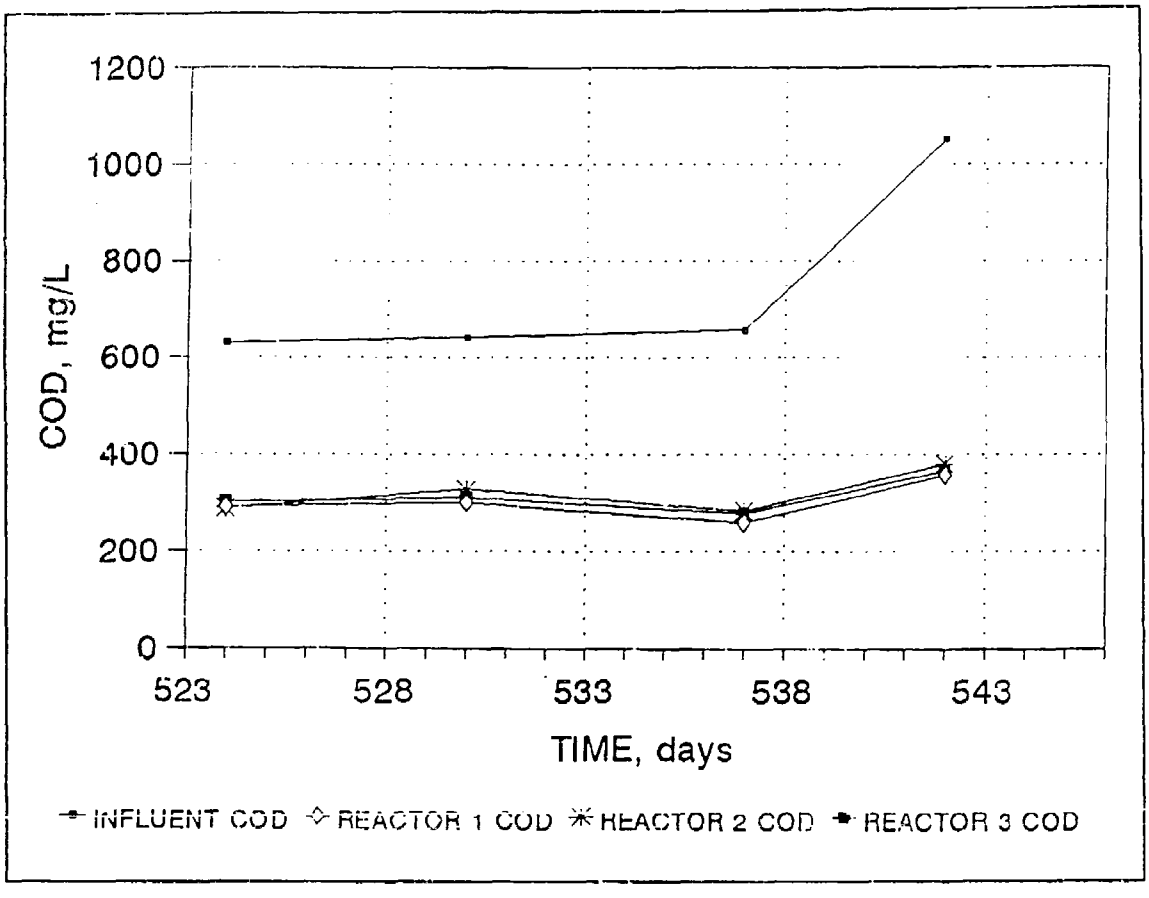



Figure 35. Optimization Phase COD data

## Economic Evaluation

To evaluate the comparative economics associated with the various oxygen sources, a pilot-scale AS system was designed based on the results of this bench study. Each oxygen source was evaluated in terms of scale-up and quotes from several equipment vendors obtained.

### System design

The pilot system design was based on results from bench studies, common wastewater design values presented by Metcalf and Eddy (1991), and characteristic values of groundwater used in this study were used in the design calculation. The following assumptions were used to determine reactor size and oxygen requirements:

*a.* Design flow rate, Q = 100 gpm

*b.* Hydraulic retention time, HRT = 16.7 hr.

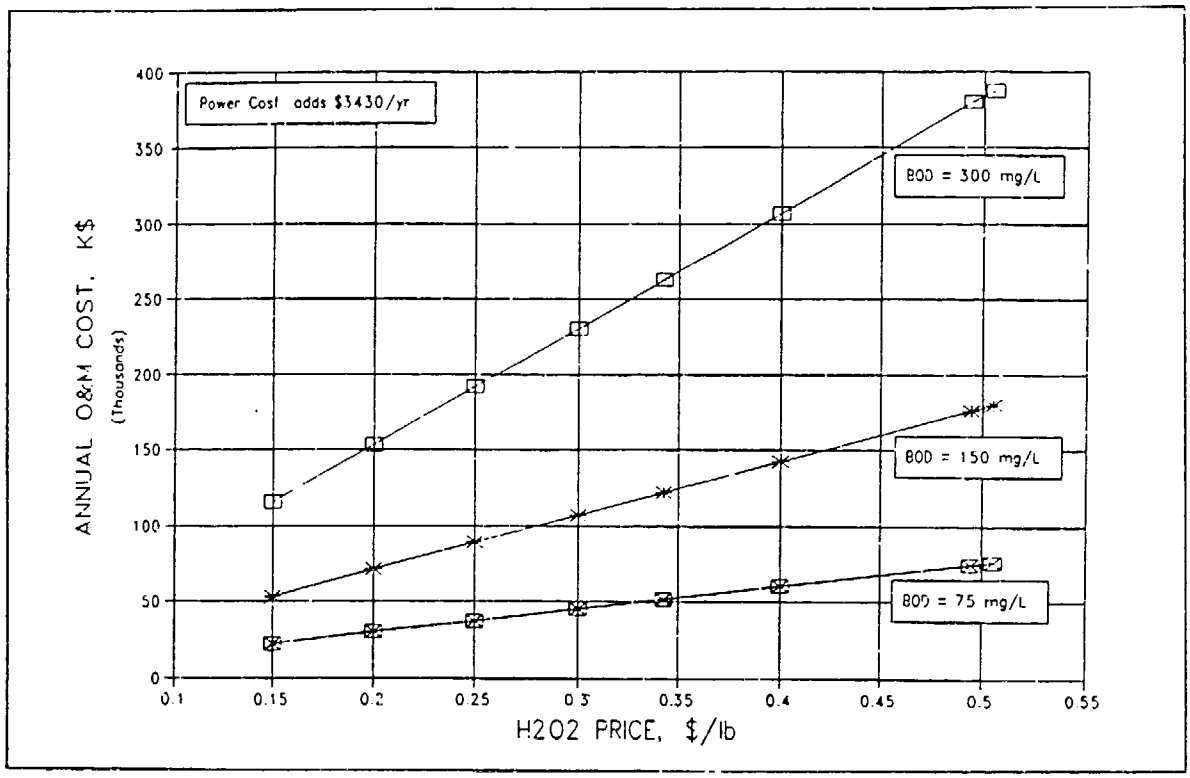



Figure 36 Cost analysis of hydrogen peroxide dosing

*c.* Volume of aeration basin, $V_R$, = 100,500 gal.

*d.* Basin dimensions = 20 by 45 by 15 ft.

*e* $BOD_5$ influent = 270 mg/ℓ.

*f.* $BOD_5$ effluent = 20 mg/ℓ.

From Metcalf and Eddy (1991), it was further assumed that $BOD_5$ = 0.68 $BOD_L$ (ultimate BOD). Also based on the results of this study, minimum sludge wasting would be required. The total amount of $BOD_5$ (in pounds) degraded in the bioreactor was calculated by.

$$\text{Mass BOD utilized} = \frac{Q\ (S-S_o)}{0.68} \times 8.34 = 442 \text{ lb BOD/day}$$

Assuming an 8-percent oxygen transfer efficiency and a design safety factor of 2, the design air requirement for the pilot system was estimated to be 440 cfm or 635,000 cfd For cost-estimating purposes, an activated carbon air treatment system was included. Disposal or regeneration costs were not included in the cost estimates presented As part of the carbon system, a process stream dehumidifier system was included because activated carbon is inefficient at relative humidities greater than 40 to 50 percent. Also, a

commercial mixer was included in the cost estimates for both the pure oxygen and hydrogen peroxide fed systems because these systems will not have sufficient ML mixing capabilities.

Comparisons assume that costs for the basin, piping, and sludge handling equipment will be approximately equal for all three schemes. Typical power and chemical costs were obtained from appropriate vendors. Power costs were assumed to be $0.07/kWhr for all systems. Calculations assume 24-hr/day, year round operation of the treatment facility and include standard engineering safety factors. Appendix C presents the cost information used in estimating the costs presented below.

### Air system

A cost estimate for an aeration system was obtained from Dr. James E. Bowie, Acadiana Treatment Systems, Duson, Louisiana. The estimated price for blowers, motor, valves, and associated piping is $22,965. Power costs for the 25-hp motor and air stream dehumidifier were $16,040 annually. Mixing of AS in the reaction vessel was designed to be accomplished via the aeration system; therefore, a mixer was not required.

### Pure oxygen system

The quote for the oxygen system was obtained from Mr. Min-Da Ho, of Praxair, Inc. (formerly Union Carbide-Linde Division), Tarrytown, New York. The quote was based on the design flow and OUR determined during this study. Approximately 500 lb/day or 182,500 lb/yr of oxygen will be required to meet the system OUR. Like the peroxide system, a mixer will be required to keep biological solids in suspension in the reactor. The capital costs for this option, including an off-gas activated carbon treatment system, are approximately $27,650 with the annual operations and maintenance (O&M) costs estimated to be $18,045.

### Hydrogen peroxide system

A quote for the peroxide system was received from Mr. Gerd Scherer, Manager of Environmental Applied Technology, Degussa Chemicals Inc., Allendale, New Jersey. The required materials included to achieve the bioreactor oxygen demand are 685,000 lb of 50-percent $H_2O_2$/year, a metering pump, and piping system. A system capital cost of $20,600 was estimated along with an annual O&M estimated cost of $274,000.

The impact of hydrogen peroxide costs on annual operations is apparent. The unit cost used in this estimate was $0.40 per pound. Further investigation into hydrogen peroxide cost indicated that actual unit costs varied dramatically with quantity and locality. Also, reducing the system organic loading (i.e.,

lower influent BOD) reduces hydrogen peroxide usage, thereby reducing the annual cost. Figure 36 presents a cost analysis of how both influent BOD and hydrogen peroxide unit cost impacts the annual O&M costs. From this figure, it can be noted that as organic loading is reduced and lower hydrogen peroxide unit prices are used, the economics associated with hydrogen peroxide usage as an oxygen source decrease. However, it is realized that lower BODs represent lower air and oxygen requirements. Subsequent inquiries on hydrogen peroxide costs indicated that hydrogen peroxide unit costs as low as $0.20 may be found under the appropriate conditions (i.e., required quantities and location to the sales facility).

## Summary

The economic analysis performed on the results of this study indicates that the hydrogen peroxide fed system had the lowest capital cost at $20,600, with the air sparged system being second at $22,965, and the pure oxygen sparged system the most capital intensive at $27,650. In terms of operations and maintenance costs per annum, the pure oxygen sparged system was cheapest at $18,045 per year, followed by the air sparged system at $23,540 per year, and the hydrogen peroxide fed system costing a full order of magnitude higher at $277,430 per year.

Another consideration is the case when activated carbon is a poor adsorbent for the VOC being treated. In ongoing studies at WES by Gunnison, Pennington, and Zappi (this information is not yet published), the determination has been made that n-nitrosodimethylamine (NDMA) does aerobically biologically degrade; however, this compound is a very poor adsorbate on activated carbon. Using activated carbon for removing NDMA from off-gases would be extremely expensive. Also, the BOD of a system treating NDMA-contaminated groundwater will likely have a very low BOD. The combination of these two factors is one of the few cases where using hydrogen peroxide as an oxygen source will be cost-effective.

In summary, hydrogen peroxide is capable of supporting an AS system. However, unless the influent VOCs are poor adsorbents, a low influent organic loading is involved, or volatile amendments, such as toluene for dioxygenase degradation of TCE, are involved, then the use of hydrogen peroxide is cost prohibitive, but technically feasible.

# 4 Conclusions

The Acclimation Phase of this study indicated that an inoculum from a suspended growth municipal sewage treatment plant could be acclimated to the contaminants in the RMA Well 01014 groundwater. However, the ability of the AS process to utilize the contaminants in the groundwater as the sole carbon sources was not evaluated. Respirometer testing of a nonacclimated microbial consortia to the acclimated consortia did indicate significant benefit toward the use of the acclimated consortia.

The AS process has potential for removing gross pollutants and VOCs from the groundwater. BOD removals were always in excess of 95 percent. However, COD removals were always in excess of only 50 percent. Benzene was never detected in the effluents from the bioreactors during the course of the study. A steady-state hydrogen peroxide fate model was proposed. This model can be used by design engineers for estimating hydrogen peroxide dosing requirements.

A bioreactor was successfully maintained using hydrogen peroxide as the sole source of oxygen. Air and oxygen sparged bioreactors served as test controls. There was no observed difference between the various bioreactors in terms of contaminant removal and biomass stability except for the benzene off-gassing. Analysis of the gas streams exiting the bioreactors indicated that approximately 15 and 1 percent of the benzene was removed from the air and oxygen sparged bioreactors via volatilization, respectively.

The cost of operating an AS bioreactor using hydrogen peroxide is approximately an order of magnitude more costly than the gas sparged systems equipped with off-gas treatment. Oxygen sparging with off-gas treatment seemed the most cost-effective technique of all by costing only 0.75 percent of the costs associated with air sparging with off-gas treatment.

In summary, the use of the hydrogen peroxide as an alternative oxygen source has potential for some select situations. The degree of process economic feasibility will depend on the influent contaminant type and concentrations requiring treatment.

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# Appendix A
# History of System Operations and Observations

## In-House Laboratory Independent Research Program Activity Summary

1/8/92 Test Day 1

Began groundwater acclimation phase of project. Reactors ran at constant conditions for 3 weeks prior to first addition of groundwater.

8/24/92 Test Day 24

Flow rate was set for 6 ml/min, giving a hydraulic retention time (HRT) of 5.56 hr. Feed consisted of the following: 4.5 g sodium acetate, 3.6 g ammonium sulfate, 0.3 g ammonium phosphate, 0.5 g dextrose, and 0.6 g glutamic acid. In addition, 150 ml of sludge was transferred from Reactor 3 to Reactors 1 and 2.

8/26/92 Test Day 26

One liter of groundwater was added to 14 ℓ of influent with the above nutrients. Groundwater concentration 6.7 percent by volume.

9/3/92 Test Day 34

Two liters of groundwater were added to 13 ℓ of influent with the above nutrients. Groundwater concentration 13.3 percent by volume.

9/6/92 Test Day 37

Airflow was reduced from reading of 2,117 ml/min to 480 ml/min.

9/11/92 Test Day 42

Four liters of groundwater were added to 11 ℓ of influent with the above nutrients. Groundwater concentration 26.7 percent by volume.

9/16/92 Test Day 47

Feed rates changed to the following: 6.0 g sodium acetate, 3.6 g ammonium sulfate, 0.3 g ammonium phosphate, 1.0 g dextrose, and 1.0 g glutamic acid.

9/28/92 Test Day 59

Feed rates changed to the following. 10.0 g sodium acetate, 3.6 g ammonium sulfate, 0.3 g ammonium phosphate, 3.0 g dextrose, and 3.0 g glutamic acid. Six liters of groundwater were added to 9 ℓ of influent with the above nutrients. Groundwater concentration 40 percent by volume.

10/2/92 Test Day 63

Twenty milliliters of activated sludge from the Jackson wastewater treatment plant (WWTP) was added to Reactors 1, 2, and 3.

10/21/92 Test Day 82

Two hundred milliliters of activated sludge from the Jackson WWTP was added to Reactor 2. Eight liters of groundwater were added to 7 ℓ of influent with the above nutrients. Groundwater concentration 53.3 percent by volume.

10/26/92 Test Day 87

Ten liters of groundwater were added to 5 ℓ of influent with the above nutrients. Groundwater concentration 66.7 percent by volume.

10/30/92 Test Day 91

Flow rate decreased to 3 ml/min. HRT = 11.1 hr.

11/4/92 Test Day 96

Flow rate decreased to 2 ml/min. HRT = 16.7 hrs.
Feed rates changed to the following: 10.0 g sodium acetate, 1.2 g ammonium sulfate, 0.3 g ammonium phosphate, 3.0 g dextrose, and 3.0 g glutamic acid.

11/16/92 Test Day 108

Thirteen liters of groundwater were added to 2 ℓ of influent with the above nutrients. Groundwater concentration 86.7 percent by volume.

11/18/92 Test Day 110

Feed rates changed to the following: 10.0 g sodium acetate, 7.2 g ammonium sulfate, 0.6 g ammonium phosphate, 3.0 g dextrose, and 3.0 g glutamic acid.

11/30/92 Test Day 122

Influent changed to 100-percent Rocky Mountain Arsenal (RMA) groundwater. Feed rates changed to the following: 10.0 g sodium acetate, 1.2 g ammonium sulfate, 0.3 g ammonium phosphate, 3.0 g dextrose, and 3.0 g glutamic acid.

Groundwater acclimation phase complete.

12/14/92 Test Day 136

Because of low feed rates of influent, excessive volatilization of benzene was occurring in the influent container. To reduce volatilization, 6.5 ℓ of influent was prepared instead of the usual 15 ℓ. Nutrient addition for 6.5 ℓ of RMA groundwater are as follows: 0.667 g sodium acetate (103 ppm), 1.764 g ammonium sulfate (271 ppm), 0.078 g ammonium phosphate (12 ppm), 0.2 g dextrose (31 ppm), and 0.2 g glutamic acid (31 ppm).

12/15/92 Test Day 137

Low vortex mechanical mixers were installed on all reactors. Mixer on Reactor 2 did not work properly.

12/16/92 Test Day 138

Remaining mixers on reactors failed. All mixers were removed and returned to manufacturer.

1/28/93 Test Day 181

Begin hydrogen peroxide acclimation phase of project.

Fifty-parts per million solution of $H_2O_2$ was added to Reactor 3. Flow rate of $H_2O_2$ was 10 ml/hr.

2/8/93 Test Day 192

Feed make up remained the same as noted on 12/14/93.

2/11/93 Test Day 195

One hundred milliliters of activated sludge from the Jackson WWTP was added to each reactor.

2/16/93 Test Day 200

Installed Lightin mixers on all reactors. Speed of all reactors was 100 rpm.

2/17/93 Test Day 201

Dissolved oxygen (DO) study performed on Reactors 1 and 3. DO study of peroxide solution also conducted.

2/19/93 Test Day 203

DO test conducted by mixing 360 ml of influent with 40 ml of 50-ppm $H_2O_2$ and monitored DO for 1 hr. DO did not change in 30 min of monitoring.

Test conducted by mixing 360 ml of mixed liquor from Reactor 3 with 40 ml of 50-ppm $H_2O_2$ in a biochemical oxygen demand (BOD) bottle and monitoring DO for 30 min. Results shown below:

Reactor 3

| Time, min | DO, mg/ℓ |
|---|---|
| 0 | 6.1 |
| 5 | 5.4 |
| 10 | 5.0 |
| 30 | 4.5 |

Reactor 1

| Time, min | DO, mg/ℓ |
|---|---|
| 0 | 7.1 |
| 5 | 6.6 |
| 10 | 6.3 |
| 30 | 5.4 |

2/22/93 Test Day 206

DO study conducted on Reactor 3 by turning off air and monitoring DO in reactor. Results as follows:

| Time, min | DO, mg/ℓ |
|---|---|
| 0 | 5 |
| 5 | 2.5 |
| 10 | 0.4 |
| 30 | 0.2 |

$H_2O_2$ flow was then increased to 40 ml/hr (Reactor 3). Air was turned back on and DO monitored for 30 min. Results are as follows:

| Time, min | DO, mg/ℓ |
|---|---|
| 0 | 2.25 |
| 5 | 4.0 |
| 10 | 4.2 |
| 30 | 4.75 |

$H_2O_2$ was then returned to 10 ml/hr and the concentration increased to 200 ppm.

2/24/93 Test Day 208

DO test conducted on Reactor 3. Air was turned off and $H_2O_2$ flow increased to 20 ml/hr. DO was monitored for 30 min. Results shown below:

| Time, min | DO, mg/ℓ |
|---|---|
| 0 | 5.4 |
| 5 | 3.1 |
| 10 | 1.4 |
| 30 | 0.0 |

3/2/93 Test Day 214

Concentration of $H_2O_2$ was changed to 1,000 mg/ℓ.
DO of Reactor 3 was 7.2 mg/ℓ.

3/3/93 Test Day 215

Airflow on Reactor 3 reduced by one-half to 240 ml/hr. DO in reactor for next 30 min was as follows:

| Time, min | DO, mg/ℓ |
|---|---|
| 0 | 4.5 |
| 5 | 3.75 |
| 10 | 3.0 |
| 30 | 2.0 |

3/4/93 Test Day 216

Flow rate of $H_2O_2$ was increased to 40 ml/hr.
Also added 55.25 g of potassium phosphate dibasic.

3/9/93 Test Day 221

Added 100 ml of settled sludge from the Jackson WWTP to each reactor.
Conducted peroxide degradation studies on 3/9, 3/10, and 3/11.

3/15/93 Test Day 227

Began adding 0.4875 g of calcium oxide (CaO) to the influent to raise pH.

3/17/93 Test Day 229

Acetate, glutamic acid, and dextrose amounts were doubled in the influent. Nutrients added to influent now as follows: 1.2 g sodium acetate (ppm), 1.764 g ammonium sulfate (271 ppm), 0.078 g ammonium phosphate (12 ppm), 0.4 g dextrose (62 ppm), and 0.4 g glutamic acid (62 ppm).

3/23/93 Test Day 235

$H_2O_2$ DO study conducted.
Changed blade in Reactor 1.

5/5/93 Test Day 278

Conducted DO time study on Reactor 3. Air was turned off and DO monitored for 30 min. Results as follows:

| Time, min | DO, mg/ℓ |
|---|---|
| 0 | 10.0 |
| 5 | 4.8 |
| 10 | 1.4 |
| 30 | 0.0 |

5/12/93 Test Day 285

Air on Reactor 3 reduced to 1,145 ml/min. (Down from 1,500 ml/min.)

5/24/93 Test Day 297

Air on Reactor 3 reduced to 699 ml/min. (Down from 1,145 ml/min.)

6/2/93 Test Day 306

Turned off all air to Reactor 3.
End of hydrogen peroxide acclimation phase.

6/25/93 Test Day 329

Begin comparison phase of project

Reactor 2 converted to pure oxygen for air supply. Initial flow rate estimated at 144 ml/min.
All reactors running on different oxygen sources.

7/20/93 Test Day 354

Conducted peroxide degradation studies with mixed liquor from Jackson WWTP. Mixed liquor was taken from settled, "contacted" sludge from the sludge aeration basin.

First Test

Used 500 ml of mixed liquor and 500 ml distilled deionized (DDI) water. Added 0.2 ml of 50-percent $H_2O_2$. Mixed peroxide with mixed liquor and timed. Took samples at 5, 10, 15, 20, and 30 min. Results from test are shown.

| Time, min | $[H_2O_2]$, ppm |
|---|---|
| 5 | 25 |
| 10 | 2 |
| 15 | 0 |

The peroxide appeared to react very rapidly with the organic material in the mixed liquor. No residual was found after 10 min.

Second Test

Used 125 ml of mixed liquor and 875 ml DDI water. Added 0.2 ml of 50-percent $H_2O_2$. Mixed peroxide with mixed liquor and timed. Took samples at 5, 10, 15, 20, and 30 min. Results from test are shown.

| Time, min | $[H_2O_2]$, ppm |
|---|---|
| 5 | 14.3 |
| 10 | 25.5 |
| 15 | 16.5 |
| 20 | 16.5 |
| 30 | 15.3 |

Third Test

Used 77.5 ml of mixed liquor and 922.5 ml DDI water. Added 0.2 ml of 50-percent $H_2O_2$. Mixed peroxide with mixed liquor and timed. Took samples at 5, 10, 15, 20, and 30 min. Results from test are shown.

| Time, min | $[H_2O_2]$, ppm |
|---|---|
| 5 | 26.35 |
| 10 | 25 (Quant Strip) |
| 15 | 16.83 |
| 20 | 2 (Quant Strip) |
| 30 | 0.5 (Quant Strip) |

10/15/93 Test Day 441

End comparison phase of project.

12/20/93

All biomass in Reactor 1 floating in clarifier section. Stirred and sludge sunk to bottom and reentered reaction section. Added 20 ml of mixed liquor from Reactor 2 to Reactor 1.

1/4/94 Test Day 523

Converted all reactors to $H_2O_2$. Air and $O_2$ on Reactors 1 and 2 remained on until conversion and partial acclimation was completed. $H_2O_2$ feed rates set to provide 10, 50, and 100 ppm to Reactors 1, 2, and 3, respectively. Flow rates set at 0.5 ml/min for all $H_2O_2$ and at 3.0 ml/min for influent.

Air and $O_2$ turned off slowly over the next several days.

1/5/94 Test Day 523

Begin $H_2O_2$ optimization phase of project.

1/19/94

Checked flow rates for influent and $H_2O_2$.
Found that influent was set at about 1.4 ml/min instead of 3 ml/min. Adjusted flow and rechecked to ensure correct setting at 3 ml/min.

1/21/93 Test Day 539

Turned air back on in Reactor 3 because of gross decline in suspended solids (SS) in reactor. Added 20 ml of mixed liquor from Reactor 2.
SS in Reactor 1 also much reduced. Increased $H_2O_2$ application rate to the same as that in Reactor 2.

1/28/94 Test Day 546

End of optimization phase.

Chemical oxygen demand readings from raw groundwater taken from storage barrel:

| Date | mg/ℓ | Description |
|---|---|---|
| 1/11/94 | 780 | raw |
| | 753 | raw |
| 1/14/94 | | |
| | 735 | raw |
| | 737 | raw |
| | 925 | w/additives |
| | 900 | w/additives |
| 1/26/94 | | |
| | 1,151 | raw |
| | 973 | raw |
| | 990 | raw |

# Appendix B
# Analysis of Hydrogen Peroxide

## Evaluation of Candidate Hydrogen Peroxide Analytical Techniques

One of the important issues to be resolved in the In-House Laboratory Independent Research Program (ILIR) study was to determine the rates of $H_2O_2$ degradation in the mixed liquor of an activated sludge system. To determine these rates, the residual $H_2O_2$ concentration in a sample is taken repeatedly over time, and a plot of $H_2O_2$ concentration versus time is obtained. Several methods for determining $H_2O_2$ concentrations in the mixed liquor were investigated.

## Standard Iodometric Method

A well-known method for determining ozone concentrations in water (ozone is an oxidant similar to $H_2O_2$) uses iodide and sodium thiosulfate as reagents. An unknown amount of oxidant reacts with potassium iodide (KI), which is in excess. The colorless iodide ions, $I^-$, in the solution are oxidized to iodine, $I_2$, which has a yellowish color. The $I_2$ immediately forms $I_3^-$ with excess $I^-$ ions in the solution. The solution is then titrated with standard sodium thiosulfate until only a faint yellow color remains. Starch is added, which complexes with the $I_3^-$, giving a deep blue color. The solution is then rapidly titrated to the clear end point, and the amount of oxidant in the sample computed. The half reactions in this test are shown below.

$$H_2O_2 + 2H^+ + 2e^- = 2H_2O$$
$$2I^- = I_2 + 2e^-$$
$$H_2O_2 + 2H^+ + 2I^- = 2H_2O + I_2$$

---

$$I_2 + I^- = I_3^-$$

---

$$2S_2O_3^{2-} = S_4O_6^{2-} + 2e^-$$
$$I_3^- + 2e^- = 3I^-$$
$$2S_2O_3^{2-} + I_3^- = S_4O_6^{2-} + 3I^-$$

## Reagents

Standard solutions of $H_2O_2$ were made with concentrations of 25, 50, 75, and 100 ppm from a 50-percent by weight solution of $H_2O_2$ to test this method in this application. The equation shown below was used to calculate the volume of $H_2O_2$ to be added to deionized distilled water (DDI) to form these concentrations.

$$V \quad H_2O_2 \text{ (ml)} = \frac{\text{ppm } H_2O \text{ desired} \times \text{volume of sample, } \ell}{720\ (1{,}440 \text{ mg}/\ell \times 0.5)}$$

A 2-percent by weight solution of potassium iodide (KI) was made by adding DDI to 23.2 g of KI to bring the volume to 1 $\ell$. This solution contributes an excess of $I^-$ to the reaction making $H_2O_2$ the limiting reagent in the reaction of this method. A 0.001 N solution of sodium thiosulfate ($Na_2S_2O_3$) was made by adding DDI to 40 ml of a 0.025 N stock solution of $Na_2S_2O_3$ to bring the volume to 1 $\ell$. A stock solution of starch indicator and a 6 M solution of HCl were also used.

## Procedure

Fifty milliliters of the KI solution was added to a flask. A few drops of HCL were added just prior to adding the sample. The sample consisted of liquor from an activated slurry reactor and an appropriate volume of $H_2O_2$ to yield the desired concentration. Ten milliliters of sample were added, and then two milliliters of the starch indicator solution were added. $Na_2S_2O_3$ was then titrated until the solution was clear. The concentration of $H_2O_2$ was calculated after the titration using the following formula:

$$[H_2O_2] = \frac{\text{Normality of } Na_2S_2O_3 \times \text{volume of titrant}}{2 \times \text{volume of sample}}$$

### Results

Several trials were conducted using this method. The concentrations of $H_2O_2$ calculated using this method deviated from those concentrations determined using hydrogen peroxide quant strips. The error of this method in this application may be attributed to the high amount of solids on an activated sludge system. The high amount of solids in this system interferes with the identification of color end points necessary for reliable results.

## Spectrophotometric Determination

Because of the unreliability of the standard iodometric method in this application, an attempt was made to determine $H_2O_2$ concentration using spectrophotometric analysis of different combinations of KI, $I_2$, $H_2O_2$, HCl, and starch solutions. The absorbance curves of these solutions were analyzed in an attempt to find linear relationships between absorbance peaks of compounds of known concentrations. From these relationships, the unknown concentration of hydrogen peroxide could be determined indirectly. The solvent used in all of the solutions was DDI. The assay developed is sensitive to $10^{-6}$ moles per liter.

The spectra of an $I_2$ solution was taken and established that the peak absorbance of $I_2$ was at about 460 nm (Figure B1). The peak absorbance of the $I^-$ ion, determined by taking the spectra of a KI solution, was below 250 nm (Figure B2). The only noticeable peak in the spectra of an $I_2$ and starch solution was at 460 nm, or in the same place, and at the same intensity as the peak of a solution of just $I_2$ at the same concentration. The spectra of a mixture of starch solution and KI solution showed a peak in the same location as a solution of KI. This implies that the starch complexes with $I_3^-$, not $I_2$, or $I^-$. As seen in the reactions presented earlier, $H_2O_2$ oxidizes $I^-$, from KI, to $I_2$ which reacts with $I^-$ in the solution to form $I_3^-$. The $I_3^-$ absorbance peaks were determined by analyzing the spectra of a solution of $H_2O_2$, starch, and KI and a solution of $H_2O_2$ and KI. The $H_2O_2$, starch, and KI solution yielded peaks at 565, 350, and 290 nm (Figure B3). The $H_2O_2$ and KI solution yielded peaks at 290 and 350 nm (Figure B4). This suggests that the $I_3^-$ ion forms peaks at 290 and 350 nm and complexes with the starch to form a peak at 565 nm. The peak formed from the absorbance of the starch complex is at the direct expense of the two $I_3^-$ peaks. Since the $I_3^-$ forms in a reaction of $I^-$ and $I_2$, each $I_2$ will be consumed if the $I^-$ is in excess and the reaction is given time to proceed to completion. As seen in the balanced equation of the reaction, there is a 1:1 ratio of $H_2O_2$ and $I_2$. If the spectra is taken of a solution containing an unknown concentration of $H_2O_2$, and excess KI, and all of the $H_2O_2$ is given time to react, the $H_2O_2$ concentration can be determined from the absorbance

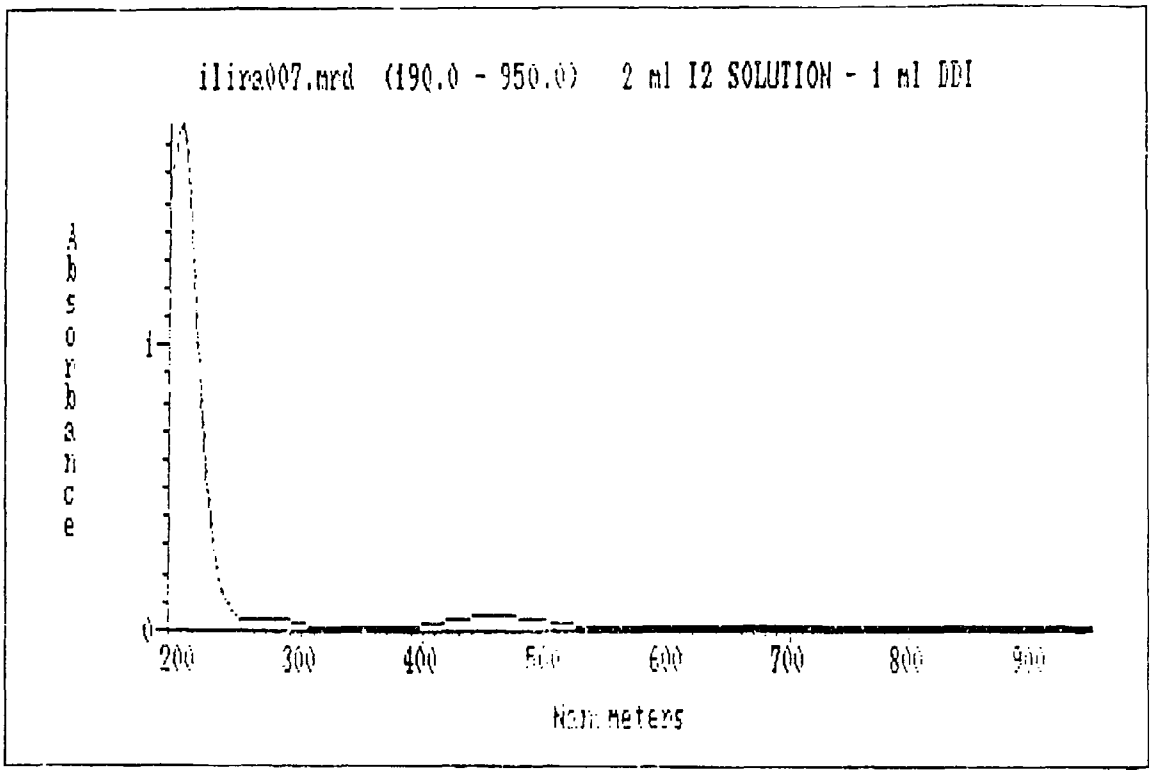


Figure B1. Absorbance of $I_2$ solution

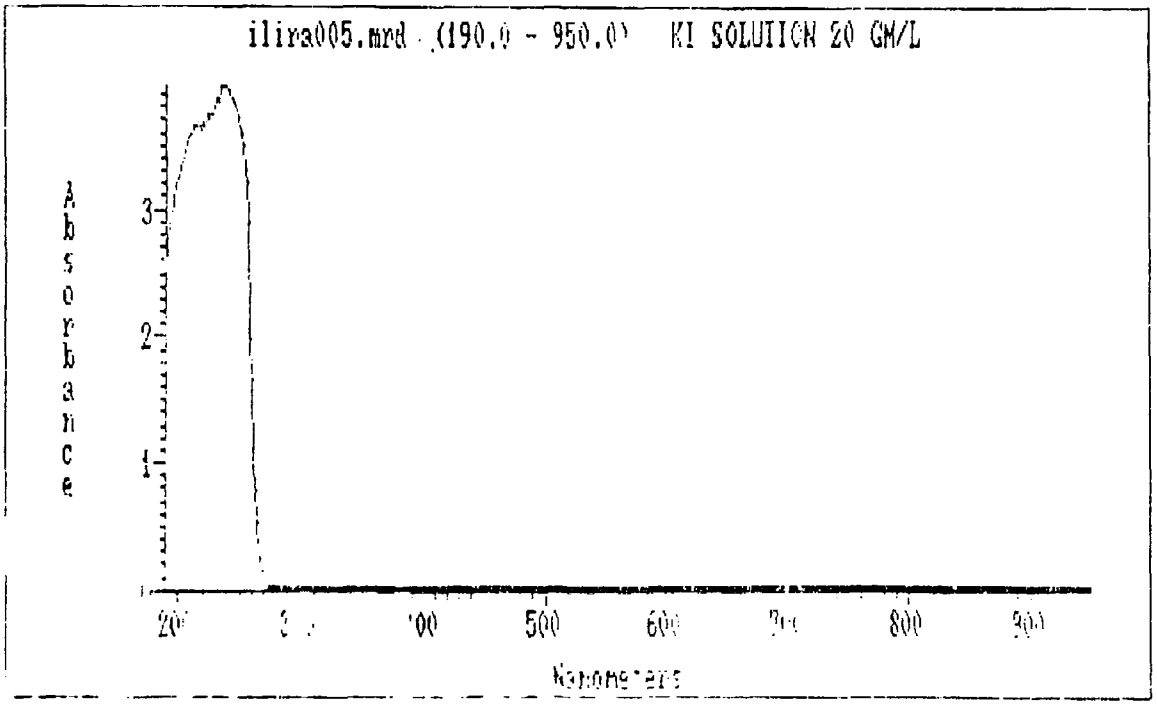


Figure B2. Absorbance of [illegible] solution

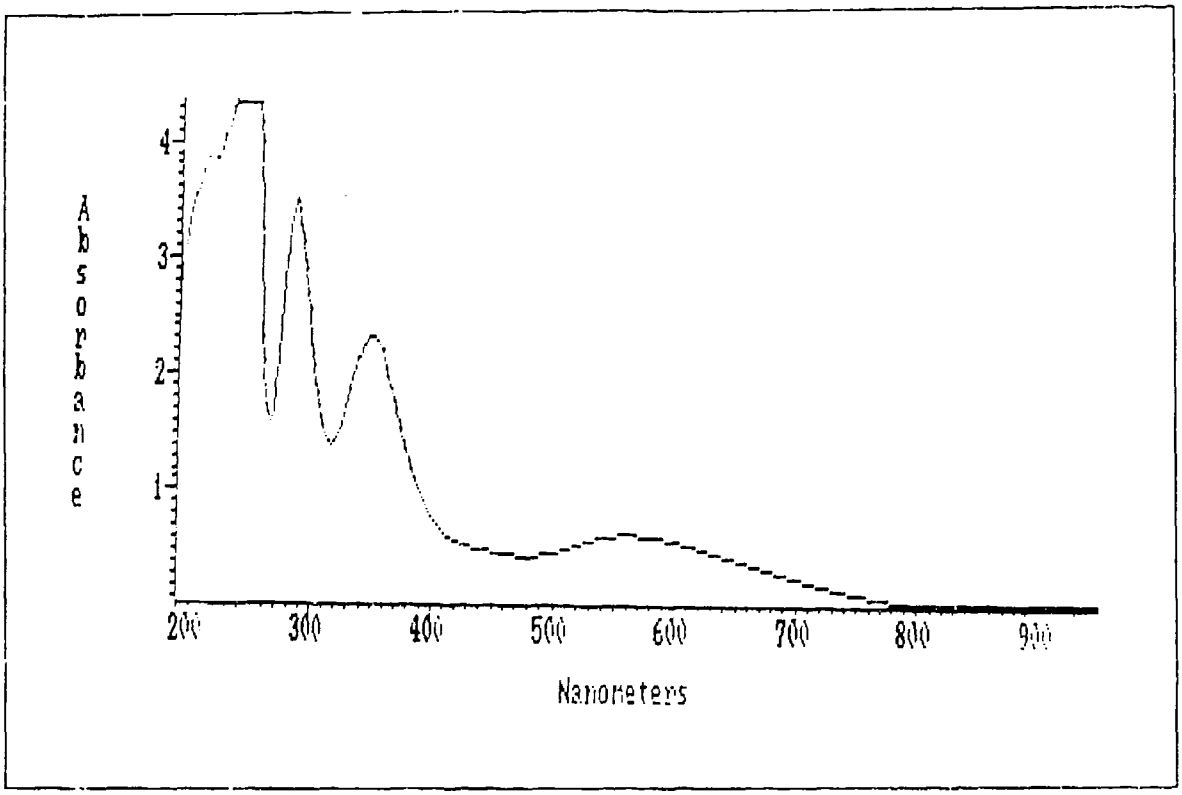


Figure B3. Absorbance of $H_2O_2$, KI, and starch solution

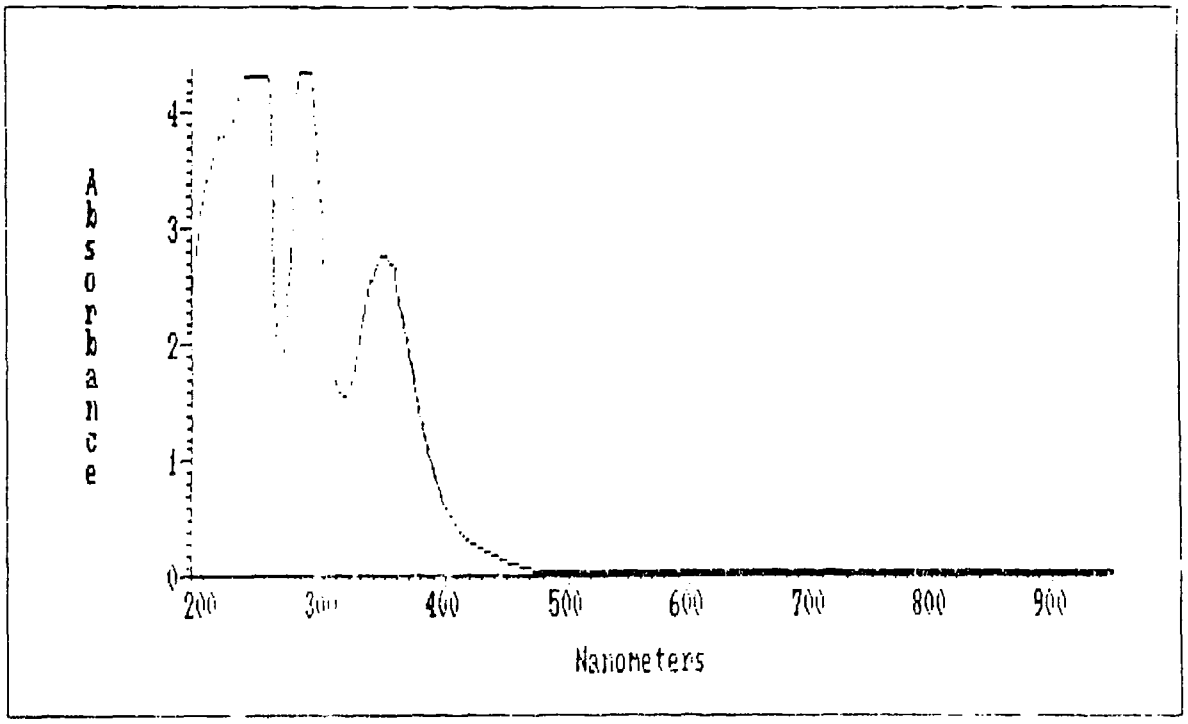


Figure B4. Absorbance of $H_2O_2$ and KI solution

curves of the starch $I_3^-$ complex are in the 565 nm range of the spectrum, and this would avoid possible interference by other absorbing organic compounds or aromatic compounds that frequently absorb in the lower ranges of the spectrum.

As seen in the equation, the reactions in this method occur in an acid medium and were discovered to proceed slowly. HCl was first added in an attempt to speed the reaction. The HCl addition was noticed to result in smaller $I_3^-$ absorbance peaks. This occurrence may be attributed to the fact that the $Cl^-$ ions in the solution act as an oxidant because of their electronegativity and drive $I^-$ ions in the solution to $I_2$. To present an acid catalyst to the reaction, tests will be performed on the acid HI in the future. It is hoped that this acid will speed the reaction and provide accurate results.

## Colormetric Test Strips

During investigation, researchers found that EM Science, an associated company of E. Merck, Inc., had developed a test. After consultation, the test kit was purchased. The test proved very simple, reliable, and was used throughout the duration of the investigation.

An RQFlex Reflectometer (EM Science Inc., Gibbstown, NJ) was used to measure hydrogen peroxide levels. This system uses a colorimetric type test to measure hydrogen peroxide residuals in the range of 0.2 to 20 mg/ℓ. Because of the limited measurement range of the instrument, the samples taken from the mixed liquor were diluted with DDI water. This was accomplished by diluting a 5-ml sample with 95 ml of DDI water for a 20:1 dilution. Results displayed on the meter were then multiplied by 20.

# Appendix C
# Cost Analysis Calculations

## In-House Laboratory Independent Research Program Economic Analysis

To evaluate the costs of the various treatment regimes, a pilot-scale activated sludge system was designed. Each oxygen source was evaluated, and quotes from several equipment vendors were obtained. The system design and cost analysis are presented here.

### System design

The pilot system design was based on the results from bench studies, common wastewater design values given in Metcalf and Eddy (1991) and characteristic values of wastewater expected in this application.[1]

The following assumptions were used to design basin sizes, oxygen requirements, etc.:

Design flow rate, Q, = 100 gpm
Hydraulic retention time, HRT, = 16.7 hr
Volume of Aeration Basin, $V_R$, = 100,500 gal
Basin Dimensions 20 by 45 by 15 ft

The average measured $BOD_5$ for the influent and effluent in the bench-scale study was used in design of the pilot plant.

$BOD_5$ influent = 270 mg/ℓ
$BOD_5$ effluent = 20 mg/ℓ

[1] References cited in this appendix are located at the end of the main text.

It was further assumed that $BOD_5 = 0.68\ BOD_L$ (ultimate BOD) (Metcalf and Eddy 1991) and that minimal sludge wasting would occur, ie., conservative assumption.

By the following equation:

$$\text{Mass biochemical oxygen demand (BOD) utilized} = \frac{Q(S-S_0)}{0.68} \times 8.34$$

$$= 442 \text{ lb BOD/day}$$

where

$Q$ = design flow rate, mgd

$S$ = influent $BOD_5$, mg/ℓ

$S_0$ = effluent $BOD_5$, mg/ℓ

8.34 = conversion factor

For example, assuming an 8-percent oxygen transfer efficiency and a safety factor of 2, the design air requirement for the pilot system is 440 cfm or 635,000 cfd. Vendor estimates of oxygenation systems are based on the above requirement of 442 lb/day or on data obtained from the bench study.

The conventional and pure oxygen systems will require treatment of contaminated off-gas streams. Activated carbon systems will treat the contaminated streams, and prices from appropriate vendors are shown.

## Cost analysis

Comparisons assume that costs for the basin, piping, and sludge handling equipment will be approximately equal for all three schemes.

Typical power and chemical costs were obtained from appropriate vendors. Power costs were assumed to be $0.07/kWhr for all systems. Calculations assume 24 hr/day, year-round operation of the treatment facility and include standard engineering safety factors. Maintenance costs are expected to be roughly equivalent in all three systems. Vendor equipment lists and other information are shown at Annex.

## Conventional air system

A cost estimate for an aeration system using conventional aeration was obtained from Mr. James E. Bowie of Water King/Acadiana Treatment Systems, Duson, Louisiana. The estimated price for blowers, motor, valves,

and associated piping is $13,700. Power costs for the system are shown below. Mixing of activated sludge in the reaction vessel is provided by the aeration system. All equipment costs are one-time capital costs, and power and carbon costs are annual recurring costs. Costs do not include carbon disposal.

| | |
|---|---|
| Equipment | |
| Aeration system | $13,665 |
| Carbon vessel | $ 4,800 |
| Heater-dehumidifier | $ 4,500 |
| Power | |
| Aeration system | $11,440 |
| Dehumidifier | $ 4,600 |
| Carbon | $ 7,500 |
| | $46,505 |

## Pure oxygen system

The quote for the oxygen system was obtained from Mr. Min-Da Ho, Praxair, Inc. (formerly Union Carbide-Linde Division), Tarrytown, NY. The quote was based on the design flow and daily oxygen demand. Approximately 500 lb/day or 182,500 lb/year of oxygen will be required. Since a much smaller volume of gas will be injected into the reaction vessel, it will not provide adequate mixing in the basin. A mixer will be required to keep bio-solids suspended in the reactor.

Some specialized dosing equipment is also required and could be rented for the prices shown below. All equipment costs shown are one-time capital costs, and power, chemical, and carbon costs are annual recurring costs. Costs do not include carbon disposal.

| | |
|---|---|
| Equipment | |
| $O_2$ application system | $ 6,000 |
| Carbon vessel (2) | $ 2,400 |
| Heater-dehumidifier | $ 3,650 |
| Mixer | $15,600 |
| Chemical | |
| $O_2$ @ $140/ton | $12,775 |
| Power | |
| Mixer | $ 3,430 |
| Dehumidifier | $ 1,840 |
| Total | $45,695 |

## Hydrogen peroxide system

A quote for the peroxide system was received from Mr. Gerd Scherer, Manager of Environmental Applied Technology, Degussa Corporation. The estimate was based on the above flow regime and data obtained during bench-scale studies for peroxide application rates. The required materials include 685,000 lb of 50-percent $H_2O_2$/year, a metering pump and piping system, and peroxide storage equipment. A mixer would be required in this system since no mixing will be provided by the oxygenation system as in the standard system. All equipment costs are one-time capital costs; power and chemical costs are annually recurring.

| | |
|---|---|
| Equipment | |
| $H_2O_2$ storage (tote bin) | $ 3,500 |
| $H_2O_2$ metering pump | $ 1,500 |
| Mixer | $ 15,600 |
| Chemical | |
| $H_2O_2$ (50 percent) @ $0.40/lb | $274,000 |
| Power | |
| Mixer | $ 3,430 |
| Total | $298,030 |

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The Department of Defense has over 12,000 sites that are contaminated from past military activities. Many sites contain volatile organic contaminants that may be treated using aboveground or in situ aerobic biotreatment systems.

Three oxygen sources for activated sludge were evaluated using 3-ℓ chemostats treating amended groundwater containing over 200 mg/ℓ benzene. The oxygen sources were compared based on percent contaminant removal, reduction of benzene off-gassing, and biomass stability. The fate of hydrogen peroxide in an activated sludge system was evaluated and described using an equation that determines steady-state hydrogen peroxide concentration. The equation is essentially a simple fate model that includes input, output, and both biotic and abiotic terms.

The results indicated that peroxide could be used as an alternative oxygen source with no release of benzene into the ambient air; however, this approach was found to be expensive because of the high cost of hydrogen peroxide. Benzene was removed from the groundwater to below detection levels. Pure oxygen sparging was a particularly attractive means of introducing oxygen with minimal stripping of benzene. Biotic reactions dominated hydrogen peroxide fate within an activated sludge system. The reaction between hydrogen peroxide and biomass was second order overall and first order with respect to both reactants.

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